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The cost-effectiveness of ESBL detection: towards molecular detection methods?

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Abstract

Correct detection of extended-spectrum beta-lactamases (ESBLs) is crucial for infection control and antibiotic choice. We performed a study to determine the cost-effectiveness of phenotypical testing, which can be inaccurate, and genotypical tests, which are considered to be more reliable but also more expensive. All patients that had been in isolation in the Amphia hospital because of the detection of ESBL according to the ESBL Etest were included in the survey. All strains were retested using the double disk confirmation test (DDCT) and a genotypical method. This was a commercially available microarray (Check-Points). Discordant results were confirmed by PCR and sequencing. In total 174 patients were included. In 24 of 174 (14%) patients, ESBL carriage could not be confirmed with the microarray. This was verified with PCR and sequencing. The mean duration of isolation was 15 days, adding up to a total number of isolation days of 2571. False-positive results according to the microarray resulted in a total of 279 days of unnecessary isolation for the Etest and 151 days for the DDCT. Using Etest to detect the presence of ESBL results in a false-positive outcome in 14% of the cases. This results in unnecessary isolation of patients, which can be omitted by using a genotypic method.

Keywords: Check-Points, cost-effectiveness, ESBL, ESBL detection, Etest, microarray
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Introduction

The prevalence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae is increasing rapidly [1–3]. Infections with ESBL are associated with increased costs and mortality [4]. The control of ESBL is difficult as the resistance genes of these microorganisms are located on plasmids and may be transferred between different bacterial species and even different genera of Enterobacteriaceae [5]. According to the Dutch guidelines for infection control all hospitalized patients colonised with ESBL have to be placed in isolation in a separate room [6]. The current national guideline for microbiological detection of ESBL in the Netherlands recommends the use of phenotypical tests for confirmation of ESBL. These tests are sometimes difficult to interpret and may lead to false-positive tests, resulting in unnecessary isolation of patients and possibly inappropriate treatment, leading to higher costs. Phenotypical testing takes at least 1 day to provide results.

Genotypical methods are considered to be more accurate, can provide characterization of the ESBL genes and have the potential to provide results on the same day.

Recently, a new diagnostic microarray for detection and identification of ESBL in Enterobacteriaceae has become available. Several studies have concluded that the diagnostic microarray is superior to phenotypical methods. Sensitivity for ESBL was comparable to or higher than phenotypic methods, whereas specificity was consistently higher [7–10]. The microarray had almost the same sensitivity and specificity as PCR with additional sequencing and was therefore used as the standard for testing [11].

We wanted to determine the effect of false-positive phenotypic testing on infection control measures and also

evaluate the cost-effectiveness of ESBL testing. This was done to determine if implementation of a genotypical test is warranted.

Methods

Patients who had been isolated because of ESBL from November 2006 until August 2010 in Amphia Hospital in Breda were included. The Amphia Hospital is a large teaching hospital located in the south of the Netherlands. The presumed ESBL-positive strains had been stored at −70°C. Information about type of organism, interpretation of phenotypical test and duration of isolation was obtained from the laboratory information system.

Primary phenotypic detection of ESBL

For species identification and susceptibility testing the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) was used. According to the Dutch national guideline for ESBL detection, classification of Enterobacteriaceae was done to the presence of chromosomal AmpC beta-lactamases in two groups. Presence of ESBL production was determined using the ESBL Etest (bioMérieux) [12]. The hands-on time and the turn-around time were, respectively, 15 min and 16 h.

Genotypic detection of ESBL

The strains that had been stored at −70°C were recollected and DNA isolation was performed using the QIAamp DNA Mini Kit system (QIAGEN, Hilden, Germany).

The ESBL Array (Check-Points, Wageningen, the Netherlands) is supplied as a kit. It is designed to detect single nucleotide polymorphisms (SNPs) of essential bla_{TEM} and bla_{SHV} variants and the following bla_{CTX-M} groups: $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-9}$ and $bla_{CTX-M-8/25}$, as described by Cohen Stuart *et al.* [7].

Microarray images are read using a microarray reader (ArrayTube Reader; ClonDiag Chip Technologies, Jena, Germany) connected to a computer running dedicated software for analysis of the images. The software indicates whether bla_{TEM}, bla_{SHV}, bla_{CTX-M} or a combination of these genes is present. The hands-on time and the turn-around time were, respectively, 2 and 8 h.

Secondary phenotypic detection of ESBL

All strains were retested using the DDCT (Rosco diagnostica, Taastrup, Denmark). Interpretation of the test was carried out according to the Dutch national guideline for the detection of ESBL in Enterobacteriaceae [12]. The hands-on time and the turn-around time were, respectively, 15 min and 16 h.

Retesting of primary phenotypical test (Etest)

After testing the strains with the microarray, the false-positive results obtained using the primary phenotypical test (Etest) were retested using the same test and the same algorithm.

Verifying the reference test (microarray)

The beta-lactamase genes were characterized by PCR at the VUmc, followed by sequencing (BaseClear, Leiden, the Netherlands), as described by Naiemi *et al.* [13]. Sequences were analysed with Bionumerics software (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium) and compared with sequences in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST) and Lahey (http://www.lahey.org/studies/).

Results

Primary phenotypical test (Etest)

In total, 174 patients were included based on the initial Etest results. Among these strains, 97% were group I Enterobacteriaceae. The organisms are shown in (Table 1). The total number of days in isolation was 2571. The average duration of isolation was 15 days (range, 1–93; median, 10).

Genotypical test

All 174 phenotypical ESBL-positive strains were retested using the microarray. The microarray detected at least one ESBL-gene in 149 strains. Among the ESBLs there were 90 $bla_{CTX-M-1}$, 33 $bla_{CTX-M-9}$, 5 $bla_{CTX-M-2-8-25}$ and 25 $bla_{TEM/SHV}$. There were four strains that contained multiple ESBL genes.

According to the microarray there were 25 false-positive phenotypical tests, resulting in a positive predictive value of 86% for the Etest. In group I Enterobacteriaceae 12% (n = 21) were discordant and showed a phenotypical positive test, whereas the genotypical test was negative. In group II Enterobacteriaceae 67% (n = 4) were discordant (Table 1).

Among the 25 false-positive results there were three patients that were tested with the same bacterial species twice. This was in different episodes of isolation. The total number of isolation days of these false-positive tests was 279 days (Table 2).

Secondary phenotypical test (DDCT)

At retesting of the 174 strains, 161 tested ESBL-positive using the DDCT. Among these 161 positive phenotypical tests are 13 false-positive tests according to the microarray.

			Discordant results			
			Etest 14%		DDCT 7%	
Group I (*n* = 168)	*Escherichia coli*	145	7	12%	3	5%
	Klebsiella (pneumoniae 14, oxytoca 7)	21	11		4	
	Proteus mirabilis	2	2		1	
Group II (*n* = 6)	*Enterobacter cloacae*	4	2	67%	2	67%
	Citrobacter freundii	2	2		2	

TABLE 1. Microorganisms found among positive primary phenotypical test (Etest). Discordant results in the phenotypical test compared with the microarray. Classification according to Group I and Group II Enterobacteriaceae

TABLE 2. Performance and costs (material only) of different diagnostic tools compared with the microarray, which was chosen as the reference test

	Cost per test	Total cost for testing (*n* = 174)	Total days of isolation	Extra isolation days
Etest	€ 25	€ 4.350	2.571	+279
DDCT	€ 4	€ 696	2.443	+151
Microarray	€ 33	€ 5.742	2.292	–

Retesting of these strains with the DDCT led to the same results.

Among these false-positive tests, eight out of 13 tests were just over the cut-off. The DDCT had no false-negative tests. The total number of isolation days of these false-positive tests was 151 days (Table 2).

Retesting of primary phenotypical test (Etest)

At retesting of the 25 strains that were false-positive with the Etest, 20 tested ESBL-positive using the Etest and one could not be interpreted because of overgrowth.

Verifying the reference test (microarray)

The results of PCR and sequencing did not show ESBL in $bla_{TEM/SHV}$ in the 25 strains where the microarray could not find any ESBL genes. The PCR for $bla_{CTX\text{-}M}$ showed PCR product in seven out of 25 strains. Then sequence analysis of the PCR product was done. In six strains, all *Klebsiella oxytoca*, the PCR product was non-specific; these were bla_{OXY} genes and no ESBL genes were found. In one *E. coli* we found $bla_{CTX\text{-}M\text{-}8}$, which is an ESBL gene.

Discussion

Comparing the results of Etest with those of the microarray, 14% (*n* = 24) were discordant. In group I Enterobacteriaceae 12% (*n* = 20) of the strains were discordant and in group II Enterobacteriaceae 67% (*n* = 4). This indicates that a larger amount of group II organisms could increase the percentage of discordant results.

As reported by others, the microarray is more accurate than phenotypical tests. However, the microarray is more expensive to perform. We quantified the number of false-positive findings in clinical practice, which enabled us to estimate the savings that can be achieved. This is based on the reduction of the number of isolation days on the wards in relation to the costs of the microarray.

In this study the total cost of retesting the 174 samples (cost materials for DNA isolation and microarray) was *c.* € 6000. The total number of days of isolation that could have been avoided was 279 days. This means that the additional costs of an isolation day should be at least 22 euro to result in a net savings. The costs of isolation may vary between hospitals or countries but are likely to be substantially higher than 22 euro in most settings. Changing to a cheaper ESBL confirmation test or not performing one at all can make further reductions in costs. This study does not provide enough data for discussion of this subject.

We decided not to include the cost of laboratory labour because this is highly dependent on throughput of tests and differences in the salaries of technicians. To do so, one could use the hands-on time of the phenotypical vs. the genotypical test, which is respectively 15 vs. 120 min.

Because of the possible limitations of the microarray compared with the gold standard, PCR and gene sequencing were carried out. We retested the 25 strains in which the microarray could not find any ESBL genes. In 24 strains, results were consistent with PCR and sequencing. In one strain the microarray showed a lack of sensitivity and did not pick-up $bla_{CTX\text{-}M\text{-}8}$. This gene is included in the kit and after retesting the microarray did pick-up the $bla_{CTX\text{-}M\text{-}8}$. This lack of sensitivity did not have a major impact on this study because both tests, DDCT and Etest tested positive for this strain and the duration of this isolation episode was only 5 days.

We also showed that the PCR has a lack of specificity for $bla_{CTX\text{-}M}$ in *K. oxytica*; therefore all results should be verified by gene sequencing. The false-positive results in the phenotypic assays are likely to be caused by hyperproduction of the chromosomal K1 beta-lactamase or by effects of other resistance mechanisms that are also inhibited by clavulanic acid, for example Enterobacteriaceae carrying wild-type beta-lactamase genes. Retrospectively, we could also determine

two misinterpretations that caused a false-positive result and one strain with overgrowth that made the test uninterpretable.

It is remarkable that the DDCT did not lead to false-positive results when testing strains of *K. oxytoca* with bla_{OXY}, whereas the Etest did. In other strains the DDCT also had fewer false-positive results than the Etest.

This indicates that the performance of the DDCT is better than the Etest but this could be biased. When the DDCT was used as the initial screening and the Etest as a confirmatory method it is possible that the Etest would have found false-positive DDCT results. The low cost and more objective reading of the DDCT warrants a further prospective evaluation of the DDCT. After retrospective correction for the false-negative microarray result, among 12 false-positive results in the DDCT there were eight tests in which the result was just above the cut-off. Changing this cut-off slightly for a positive test could result in a better performance but this should be evaluated in a prospective clinical study.

A major drawback of the microarray is that it is not capable of picking up new and unknown ESBL genes that have not been incorporated in the microarray. When genotypic assays are to be implemented on a larger scale this should be accompanied by prospective surveillance for the emergence of new resistance mechanisms.

In conclusion, the currently used phenotypical tests to detect ESBL have a relatively high rate of false-positive results. The microarray provides more reliable results and thereby avoids unnecessary isolation days. In our study the additional cost of 1 day of isolation had to be at least € 22 for the microarray to be cost-effective and costs can be further reduced by changing phenotypical confirmation. Also the microarray can provide results more rapidly than phenotypic tests. Therefore, the microarray should be considered for implementation in the routine diagnostic laboratory.

Transparency Declaration

The authors declare no conflict of interest of any nature.

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