DETECTION OF EXTENDED-SPECTRUM \( \beta \)-LACTAMASES IN ENTEROBACTERIACEAE WITH COMBINATION DISK METHOD.

H. Lahdibi Sahraoui\(^1,2\), El H. Berny\(^1\), A. Quasmaoui\(^2\), R. Charof. \(^2\), Z. Mennane\(^2\)

1) Laboratory of biotechnology, environment and quality (LABEQ), Department of biology, Faculty of science, University Ibn Tofail, Kenitra, Morocco.

2) Department of medical bacteriology, National Institute of Hygiene, Rabat, Morocco.

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Abstract

ESBLs are now a problem in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates varies greatly worldwide and in geographic areas and are rapidly changing over time. A total of 81 isolates of Enterobacteriaceae ESBLS, have been chosen in order to confirm ESBL-producing isolates, this confirmation was done by inhibitor potentiated disc diffusion test. Of these 77(95.06%) showed increase in zone diameter (≥5mm). This study finds Combined Double Disc Test to be the reliable phenotypic method for ESBLs detection.

Keywords: ESBL, combined disk, ceftazidime-clavulanic acid, Enterobacteriaceae.

INTRODUCTION

Gram negative bacteria are the common pathogens causing wide spread infections, both nosocomial and community acquired. In the Gram negative bacteria, one of the important mechanisms of resistance is production of \( \beta \)-lactamases \(^1\). There are more than 340 different beta lactamasas so far identified and the growth spurt shows no signs of slowing \(^2\).

Since their first description more than twenty years ago, pathogens producing extended-spectrum beta lactamasas (ESBLs) have become an increasing cause of clinical concern for several reasons \(^3-4\). First, systemic infections due to ESBL-producing Enterobacteriaceae are associated with severe adverse clinical outcomes. Second, initially restricted to certain geographical areas, these enzymes have spread globally and their prevalence varies by geographic region. Finally, besides the growing species diversity, ESBL phenotypes have become more complex due to the production of multiple enzymes \(^5\).

ESBLs are \( \beta \)-lactamases capable of conferring bacterial resistance to penicillins, first, second, and third-generation cephalosporins, and aztreonam (but not cephamycins or carbapenems) but are inhibited by \( \beta \)-lactamase inhibitors such as clavulanic acid. Plasmsd responsible for ESBLs production frequently carry genes encoding resistance to other drug classes also (e.g. aminoglycosides). Therefore, antibiotic options for ESBLs producing organisms are limited. Carbapenems are the treatment of choice for serious infections due to ESBLs producing organisms, yet carbapenem resistant isolates have recently been reported.

ESBLs represent an impressive example of ability of gram-negative bacteria to develop new antibiotic resistance mechanisms in face of the introduction of new antimicrobial agents \(^6\). Therefore infections due to ESBL isolates continue to pose a challenge to infection management worldwide \(^7\).

ESBLs have serine at their active sites which attack the amide bond in the lactam ring of antibiotics causing their hydrolysis. These enzymes which now number more than 150 were initially limited to \textit{Escherichia coli} and \textit{Klebsiella} species. Lately many have been spreading and are engulfing other genera specially \textit{Enterobacter} and \textit{Proteus}. ESBL phenotypes and detection have become more complex due to the diversity of the enzymes produced, emergence of inhibitor resistant ESBL variants plasmid borne resistance genes, Concurrent Amp-C production enzyme hyper production and porin loss \(^8\).

Currently, detection of ESBLs in Enterobacteriaceae is still considered useful \(^9\) or even mandatory \(^10\) for epidemiological and infection control purposes.

The introduction of the third generation cephalosporins was very much helpful in fighting against the beta-lactamasas in clinical practice \(^11\).

CLSI recommends performing phenotypic confirmation of potential ESBL-producing isolates of \textit{K. pneumoniae}, \textit{K. oxytoca}, or \textit{E. coli} by testing bothcefotaxime and ceftazidime, alone and in combination with clavulanic acid. Testing can be performed either by the broth micro-dilution method or by the disc diffusion method \(^12\).

MATERIAL AND METHODS

Bacterial strains

A total of 81 isolates of Enterobacteriaceae ESBLs from various samples of urine, blood, pus, came from five regions (A, B, C, D, E) of the medical care units, Surgery, Gynecology and Obstetrics, and Pediatrics, have been chosen in order to confirm ESBL-producing isolates, this confirmation was done by inhibitor potentiated disc diffusion test. The study included patients of all ages and both sexes. The isolates were identified by following the standard laboratory procedure.

Combination disk method

Several manufacturers have developed ESBL detection tests based on the combination disk method. The principle of this method is to measure the inhibition zone around a disk of cephalosporin and around a disk of the same cephalosporin plus clavulanate. Depending on the disk type, a difference of ≥ 5 mm between the two diameters (i.e., corresponding to a two-fold dilution), or a zone expansion of 50% are considered as indicating ESBL production \(^13, 14\). The test is easy to perform and its interpretation is straightforward. Sensitivity and specificity for this method were first reported to be 96% and 100%, respectively \(^15\).

RESULT AND DISCUSSION

ESBLs are now a problem in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates varies greatly worldwide and in geographic areas and are rapidly changing over time \(^16\). Combination disk method is easy to perform and its interpretation is straightforward. Sensitivity and specificity for this
method were first reported to be 96% and 100%, respectively [13].

Out of 81 isolates of Enterobacteriaceae ESBLs, 77 (95.06%) were positive when tested with ceftazidime-clavulanic acid combined disk and ceftazidime alone.

In line with previous reports, our study confirmed the higher sensitivity of ceftazidime/clavulinate discs (Cac) for the detection of ESBLs among Enterobacteriaceae.

In a previous work, we were compared four methods for ESBL detection; the results were mentioned in table 1.

Table(1): Showing comparison of ESBL producers by different methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Number Positive(n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>double disk synergy test (30mm)</td>
<td>17</td>
<td>20.99</td>
</tr>
<tr>
<td>double disk synergy test (25 to 30 mm)</td>
<td>73</td>
<td>90.12</td>
</tr>
<tr>
<td>double disk synergy test (20mm)</td>
<td>73</td>
<td>90.12</td>
</tr>
<tr>
<td>combination disk method</td>
<td>77</td>
<td>95.06</td>
</tr>
<tr>
<td>Double disk test (Spanish test)</td>
<td>81</td>
<td>100</td>
</tr>
</tbody>
</table>

Comparing the last two methods (combination disk method, Double disk test (Spanish test)) we can say that even if (Spanish test) was used to detect all ESBL but the practice of this technique remains somewhat difficult; because it takes time, caution should be exercised to avoid contamination. With respect to the method of combination disk method; it is easy for the clinician in his practice as well as for reading the results.

A study from Rupinder Bakshi et al. showed that ESBL positivity by combination disk method (55%) is more as compare to double disc approximation test (42%). ESBL production was observed in 48% (118/246) of E. coli, K. pneumoniae 44% (40/91) and P. aeruginosa 50% (10/20) isolates by combined disk method [17].

It was many studies confirmed that Combination disk test was effective to detect ESBL; A study done by Shukla and al found CDT (Combination disk test) to be more effective in detecting the ESBLs than DDST (Double Disc Synergy Test). DDST lacks sensitivity because of problem of optimal disc space and correct storage of clavulanate containing discs. Comparison of methods employed for detection of ESBL shows that DDST was less sensitive than CDT [18]. Mangaiyarkarasi T. et al reported that out of 138 isolates tested for ESBL production by both methods, CDT detects 71 (84.5%) whereas DDST detects 52 (61.9%) as ESBL producers [19]. A similar study was conducted by Yazdi M. In which the combined disk test done on 116 isolates resistant to third generation Cephalosporins also showed that 109 isolates were ESBL producing strains so this test can detect 94% [20]. In one study, the combined disk test detected all the ESBL isolates (n = 57). It was able to pick up one more isolate of S. Typhi as ESBL that other methods failed to identify. Using this technique, ceftazidime with ceftazidime-clavulanate achieved the highest sensitivity of 93.44%, and the highest specificity (100%) [21].

In line with previous reports, according to Yves De Gheldre et al. ceftazidime/clavulanate discs (CD02) confirmed the higher sensitivity compared with cefotaxime/clavulanate discs (CD03) for the detection of TEM- and SHV type ESBLs among E. coli and Klebsiella spp. isolates [22].

In our study, Out of 77 ESBL producing isolates, 49% (n = 38) where Escherichia coli, and 40% (n = 31) were Klebsiella pneumonia (figure1), which is similar to a study done by (Alipourfard et al. 2010) they found that 60% were E. coli and 40% were K. pneumoniae [23]. A study done by Archana Sharma et al. found where E. coli is the leading producer of ESBL followed by Klebsiella [9]. Güzel M. et al. in 2015 found that hundred and five strains (81 Escherichia coli, 24 Klebsiella spp.) were found to produce ESBL by combined disc method [24].

**Fig(1)** Distribution of Enterobacteriaceae species and their percentage

Certain microorganisms may produce more than one beta-lactamases concomitantly or may express the same beta-lactamase at high amounts, which can lead to the development of resistance also against beta-lactamase inhibitors [25].

**CONCLUSION**

In conclusion, it is very important for the clinical microbiology laboratories to have the ability to detect and report ESBLs production in clinical isolates of Gram-negative bacteria. This study finds Combined Double Disc Test to be the reliable phenotypic method for ESBL detection.

Routinely used disc diffusion susceptibility methods fail to detect ESBLs because these enzymes exhibit wide spectrum of substrate specificity. Sometimes they show false susceptibility zone of inhibition in Kirby Bauer disc diffusion test. Quick detection of ESBL strains is important since they become resistant to available antibiotics and they also pass the gene to other strains. Use of only one disc combination might fail to detect ESBL production resulting in under reporting of prevalence. Hence inclusion of more than one indicator drugs in the screening tests is recommended. Phenotypic confirmatory disc diffusion test is a simple and cost effective method for the detection of ESBLs.

**References**