Detection of ESBL and/or plasmid-mediated AmpC β-Lactamases in Entrobacteriaceae using MASTDISCTM 1D AmpC and ESBL Detection Discs and to compare this with current laboratory detection methods.

David Lorenz1, Damien Stark2, Jock Harkness3, Debbie Marriott1

1 St. Vincent’s Hospital, Department of Microbiology, Sydney, Australia.

INTRODUCTION

The β-lactamases are the most significant group of enzymes involved in conferring resistance to β-lactam antibiotics in pathogenic bacteria. They work by hydrolysing the β-lactam bond of any number of substrates thus rendering the antibiotic ineffective. Within months of the broader-spectrum β-lactam ampicillin being released in Europe in 1964, the first incidences of resistance to ampicillin in Escherichia coli was described. Today there are many β-lactam antibiotics and many more β-lactam enzymes, including Extended Spectrum β-Lactamases (ESBL’s) and plasmid-mediated AmpC β-Lactamases.

Extended Spectrum β-Lactamase (ESBL) enzymes were discovered in the early 1980’s and the use of the first dedicated ESBL test was recorded in 1987 to aid in the correct resistance patterns that extended beyond the broad-spectrum β-lactam antibiotics. Traditionally ESBL’s have been defined as enzymes that are inhibited by clavulanic acid and that have activity against extended spectrum cephalosporins. They are classified by the Ambler structural classification as molecular class C and by the Bush-Jacoby-Medeiros functional classification as functional class 2c. ESBL’s generally confer resistance to penicillins, cephalosporins and monobactams but not hydrolyse the cephamycins (ceftoxitin and cefetanite) and are inhibited by β-Lactamase inhibitors combinations such as clavulanic acid, sulbactam and tazobactam.

AmpC β-Lactamases first appeared in the late 1970’s as an inducible resistance in organisms that would overproduce their chromosomal ampC gene, probably due to the use of cephamycins and the introduction of β-lactamase inhibitor combinations. These enzymes are known as derepressed mutants and mainly form the group often referred to as ESBL’s. Escherichia coli and Shigella sonnei contain a chromosomal ampC gene but due to a lack of the regulatory gene ampR, the ampC gene is not expressed in amounts large enough to confer resistance. AmpC β-lactamases are usually resistant to penicillins, including the cephamycins and monobactams, are resistant to β-lactamase inhibitor combinations but are usually sensitive to the carbapenems. AmpC β-lactamases are Ambler molecular class C and Bush-Jacoby-Medeiros functional class 1.

The first plasmid-mediated AmpC-β-lactamase was isolated in 1988 from a Klebsiella pneumoniae that was also ciprofloxacin-resistant. Klebsiella are likely sources of plasmid-mediated AmpC-β-lactamases and are good for identification as they lack a chromosomal ampC gene. Escherichia coli, although a likely source also, is problematic as an in vitro low-level ampC expression can become hyper produced. Currently, there are over 40 known plasmid-mediated AmpC-β-lactamases derived from Enterobacter cloacae, Morganella morgani, Hafnia alvei, Citrobacter freundii and other unknown sources. The chromosomal ampC gene of E.coli has not been found on a plasmid or other transferable element and thus allows hyper production of the E.coli ampC gene by being distinguished from an E.coli with a plasmid-mediated AmpC-β-lactamase. The plasmid-mediated AmpC-β-lactamases confer resistance similar to their chromosomal counterparts.

METHODS

The ESBL’s and AmpC-β-lactamase detection methods include an insusceptibility screen using a cephalosporin (ceftaxitin) disc. This is not 100% accurate as reduced outer membrane permeability can also cause insusceptibility. Also the Amp-C Class (AC) β-lactamase plasmid-mediated AmpC-β-lactamases, are susceptible or weakly insusceptible to cefoxitin and will not be detected by the screen. Cefoxitin is an effective inhibitor of AmpC-β-lactamases and thus can be used as a substitute for cephalosporins in the DDST. The problem arises, as with the standard test, that ineffective spacing of discs can lead to incorrect results. A plasmid-mediated AmpC-β-lactamase Multiplex PCR has been described but is limited to current known transferable AmpC genes.

The aim of this study was to evaluate MASTDISCTM 1D AmpC and ESBL detection discs with current laboratory detection methods. The implications of incorrect diagnosis of either ESBL’s or plasmid-mediated AmpC-β-lactamases are far-reaching. Incorrect treatment and treatment failure can occur as well as a failure to isolate a potential infection control risk for a health care setting.

RESULTS

Both MASTDISCTM 1D ESBL Detection Discs and MASTDISCTM 1D AmpC and ESBL Detection Discs use a 5 mm increase in a combination disc plus inhibitor compared to a cephalosporin only disc as an indicator of an ESBL and/or AmpC.

19 out of 39 non plasmid-mediated AmpC E.coli’s showed an increased zone size of disc 3 of AmpC Detection Discs ranging from 1mm to 8mm. This result is unexpected and not consistent with disc instructions. Of the 13 ESBL resistant organisms, 3 E.coli’s, 3 Klebsiella spp., 6 Enterobacter spp., 1 Serratia spp and 1 Proteus mirabilis were ESBL detected by MASTDISC 1D ESBL Detection Discs and not MASTDISCTM 1D AmpC and AmpC Detection Discs.

DISCUSSION & CONCLUSION

Of the 64 ESBL’s in total, the MASTDISCTM 1D ESBL and AmpC Detection Discs (ESBL/AmpC) discs detected 63. The ESBL not detected by the ESBL/AmpC discs had an increase of 5mm to Cefazolin 30µg (CAZ) in the MASTDISCTM 1D ESBL Detection Discs (ESBL discs). The ESBL/AmpC discs not contain CAZ, only Cephaloridine 10µg (CPO). This is most likely the reason why there were possibly more cephalosporins could be developed for these discs.

The ESBL/AmpC discs detected 98% of ESBL’s and out-performed all other methods of detecting ESBL’s in the laboratory by at least 4%. The ESBL discs detected 94% of ESBL’s. The DDST’s and the CST plates did not detect any ESBL’s that the ESBL/AmpC discs didn’t detect. These screens are good indicators of ESBL’s on primary testing.

Of the 11 plasmid-mediated ESBL’s detected all but 1, an E.coli, were Cefoxitin resistant. This is perhaps an ampC hyper-producing E.coli, to properly identify transferable resistance opposed to hydro-production a multiplex PCR for plasmid-mediated AmpC genes is required. Low-level expression of E.coli’s chromosomal ampC gene is the most probable reason for seeing increased zone sizes to discs 1-3 of the ESBL discs. As long as users are aware of this and understand each E.coli, it can be worked around.

For ESBL/AmpC disc resistance as an easy means of detecting outer-membrane permeability.

When AmpC’s were not detected in the ESCHAPP’s it is perhaps due to the ampC gene not being induced (expressed) and therefore the ESBL/AmpC discs don’t detect them.

Since AmpC-β-lactamases are found chromosomally on many Entrobacteriaceae as well as on transferable elements it is very important to correctly identify the organism before calling an AmpC-β-lactamase plasmid-mediated.

REFERENCES