Technical Note

Detection of plasmid-mediated *amp*C β-lactamases using the Philisa® *amp*C ID Kit for identification of antibiotic resistance in gram-negative pathogens

Christopher M. Connelly, PhD[1], Stacey Morrow, MS, MT(ASCP)[2], Laura R. Porter[1], and Nancy D. Hanson, PhD[2]

Overview

AmpC β-lactamases are clinically important cephalosporinases that are resistant to most β-lactam antibiotics. AmpC enzymes are chromosomally encoded in many bacterial species and can be inducible and overexpressed as a consequence of mutation. Overexpression can lead to resistance to most β-lactam antibiotics. The occurrence of transmissible plasmids with acquired genes for AmpC β-lactamases often result in increased β-lactamase production, compared to chromosomally-expressed *amp*C genes. Additionally, plasmid-mediated AmpC β-lactamases can appear in organisms lacking or having low-level expression of a chromosomal *amp*C gene. Resistance due to plasmid-mediated AmpC enzymes can be broad in spectrum and often hard to detect. As such, it is useful to identify and discriminate between plasmid-mediated and chromosomally expressed AmpC β-lactamases. The Philisa® *amp*C ID Kit is a PCR-based molecular test that allows for multiplex identification of clinical isolates from six plasmid-mediated *amp*C gene families: MOX, DHA, ACC, EBC, FOX and CMY (see Table 1 for expanded list). An endogenous internal control is also included to reduce false negatives; it targets a conserved region common in gram-negative bacteria. Agarose gel detection is used to resolve PCR products and compare clinical samples against the external DNA controls. The Philisa *amp*C ID Kit can detect both plasmid-mediated and chromosomal *amp*C genes if the genes are not from the same chromosomal origin.

Table 1
Genes Identified with respective *amp*C ID primer sets

Primer Set	Gene(s)
MOX	MOX1-4, MOX8, CMY1, CMY8-11
ACC	ACC1, ACC2
FOX	FOX1-2, FOX4-9
DHA	DHA1, DHA2
CIT (CMY)*	CMY2, 4, 6, 7, 14-16, 18, 22, 25-44, 49, 53-56, 59, 60-62
EBC	ACT1-2, 8, 13 MIR1-3, 6-8
IC**	16S rRNA

* CIT refers to CMY-2-like genes that have their origin from *Citrobacter freundii*. Referenced as CMY in text.

** Internal control sequences are designed to detect *E. coli*, *Klebsiella* spp. and *Samonella* spp.

The Philisa *amp*C ID Kit has been validated by extensive testing using previously characterized clinical isolates with the Philisa® Thermal Cycler and Philisa*FAST*™ DNA Polymerase. Total PCR run time for this kit is 15 minutes, including hold times; the Philisa *amp*C ID Kit can rapidly screen test samples for the indicated gene families associated with antibiotic resistance. Optimization and validation studies demonstrated the Philisa *amp*C ID Kit's reliability when using Philisa*FAST* and the Philisa Thermal Cycler. However, the Philisa *amp*C ID Kit is compatible with most DNA polymerases and thermal cycler platforms.

Materials and Methods

Testing of the Philisa *amp*C ID Kit was done at Creighton University School of Medicine, Department of Medical Microbiology and Immunology, Center for Research in Anti-Infectives and Biotechnology (C.R.A.B.). Data was provided with permission by Dr. Nancy Hanson.

PCR Amplification:

The Philisa *amp*C ID Kit (Catalog No.: 250026), Philisa Thermal Cycler (Catalog No.: 250000), Philisa*FAST* DNA Polymerase (Catalog No.: 250024), and associated reagents were obtained from Streck, Inc. (Omaha, NE USA).

PCR was carried out as per the manufacturer's instructions for the Philisa *amp*C ID Kit: Hot start of 98 °C for 30 seconds, followed by 30 cycles [98 °C for 5 seconds, 58 °C for 10 seconds, and 72 °C for 7 seconds], and a final extension of 72 °C for 10 seconds. Rapid thermal cycling was carried out on the Philisa and the Bio-Rad C1000 Touch™. PCR amplicons were stained with ethidium bromide and resolved on a 2.5% agarose gel. A Molecular ImagerH Gel Doc XR+ System with Image Lab Software was used for PCR band detection and imaging.

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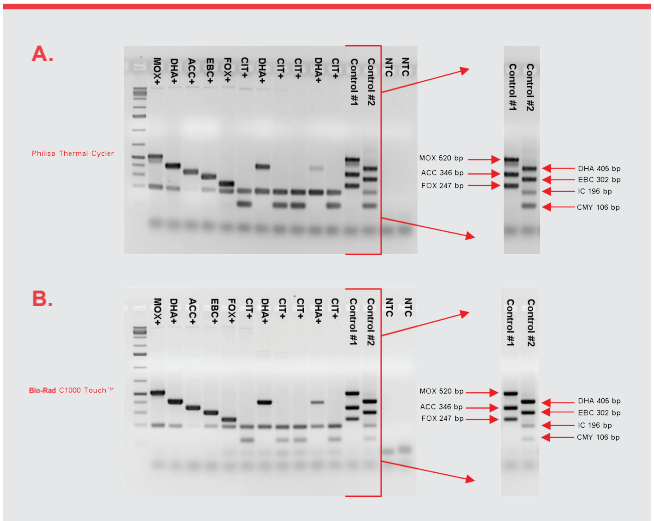
Figure 1



Detection of plasmid-mediated *amp*C β-lactamases using the Philisa *amp*C ID Kit.

Gel images depict PCR products from the Philisa *amp*C ID kit controls as well as previously characterized test isolates. (A) PCR amplification using the manufacturer's recommended protocol for rapid PCR with the Philisa Thermal Cycler and Philisa*FAST* DNA Polymerase. (B) PCR amplification using the manufacturer's recommended PCR cycling protocol for rapid PCR with the Bio-Rad C1000 Touch and Philisa*FAST* DNA Polymerase. As indicated, control #1 gel products include MOX, ACC, and FOX *amp*C gene families. Control #2 gel products include DHA, EBC, internal control, and CMY (CIT+ with test isolates) gene families.

Results and Discussion

To validate the Philisa *amp*C ID Kit, 300 previously characterized clinical isolates were tested using Philisa*FAST* DNA Polymerase with the Philisa Thermal Cycler and Bio-Rad C1000 Touch. Purified DNA from previously characterized clinical isolates was tested in this study, which included samples originating from *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* aeruginosa, *Acinetobacter* spp., *Hafnia alvei*, *Proteus mirabilis*, *Enterococcus faecium*, and *Salmonella* spp. As a demonstration of the optimized kit, Figure 1A illustrates agarose gel data from typical control PCRs (see sidebar indicating control #1 and control #2) using the Philisa *amp*C ID Kit when tested with the indicated clinical isolates per Streck's recommended instructions for use. The PCR results from this assay clearly indicate PCR amplification and successful resolution of amplicons for the indicated *amp*C families and the internal control. To identify samples testing positive for their respective *amp*C family member, the resolved amplicon sizes of the test isolates are compared to the matching control amplicon on the same gel. Using the key provided in the Philisa *amp*C ID Kit instructions for use, a match to the specified *amp*C β-lactamase gene family can be determined. Furthermore, Philisa *amp*C ID Kit testing of the same sample set on a different thermal cycler platform, the Bio-Rad C1000 Touch, demonstrated similar results (Figure 1B). However, the Philisa Thermal Cycler had the shortest PCR cycling time, producing results in ~15 minutes compared to the 32-minute* assay time for use of the Philisa *amp*C ID Kit with the Bio-Rad C1000 Touch. Although the Philisa *amp*C ID Kit performs optimally with Streck's thermal cycler and DNA polymerase, our collective studies demonstrate the kit can be used with alternative PCR platforms and DNA polymerases. Validation testing of the Philisa *amp*C ID Kit with Philisa*FAST* using the parameters described above indicated 100% sensitivity and specificity.

Conclusions:

When coupled with the Philisa Thermal Cycler, the Philisa *amp*C ID Kit facilitates sensitive and specific detection of antibiotic resistant plasmid-mediated *amp*C β-lactamases and decreases the overall time-to-results for the laboratory. Appropriate use of the Philisa *amp*C ID Kit can provide valuable information for active surveillance of antimicrobial resistance; this includes tracking changes to resistance patterns important to public health in the prevention of outbreaks and guiding the selection of the most effective antibiotic regime.

1. Research and Development Division, Streck Inc., 7002 S. 109th Street, Omaha, NE 68128. Electronic address: cconnelly@streck.com
2. Center for Research in Anti-Infectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178. Electronic address: ndhanson@creighton.edu.