Rapid Detection of Microbial Resistance to Lactam Antibiotics by LC-MS/MS

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Bacterial sepsis and septic shock are major causes of mortality worldwide. In the US it is estimated that 250,000 patients a year develop life threatening infections with a mortality rate that varies from 28 to greater than 50% depending upon other underlying disease conditions and the severity of infection (1). MALDI-TOF mass spectrometry has revolutionized bacterial identification based on patterns of ribosomal protein expression (2). The determination of bacterial resistance to antibiotics by conventional turbidometric, spectrophotometric or disk diffusion methods which evaluate bacterial growth in the presence of antibiotics is still a relatively slow process which often requires 12-24 hours of incubation. This process often delays the administration of targeted antibiotics. We have adapted a rapid screening process for identification of bacterial resistance to antibiotics by utilizing LC-MS/MS to quantitate concentrations of parent drugs and also detect hydrolysis products which result from beta-lactamase activity. The susceptibility testing can be accomplished in time periods as short as 90 minutes which includes incubation of bacteria with antibiotics and LC-MS/MS analysis. The antibiotics can be multiplexed for incubation with bacteria to minimize analysis time. We have evaluated 23 different strains of E. coli by this method including ATCC reference (3) as well as clinical isolates (20) and achieved complete concordance with traditional methods. To date the following antibiotics have been tested: penicillin, ampicillin, amoxicillin, cloxacillin, piperacillin/tazobactam, and cefotaxime. All incubations are conducted in the absence and presence of tazobactam which acts as a control. LC-MS/MS analysis was conducted on a SCIEX 3200 QTRAP® system utilizing positive ion electrospray with MRM detection and drug separation on a C18 reverse phase column using a linear methanol gradient. A sample chromatographic profile is attached.

References:

1) Dellinger R.P. et al, Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: Critical Care Medicine 2008; 36: 296-327
2) Weiser A. et al, Microbiological Diagnostics – Identification of micro-organisms and beyond: Appl. Microbiol. Biotechnol. 2012; 93: 965-974

FIGURE 1: Rapid Detection of Antibiotic Resistance Using LC/MS/MS

Clinical *E. coli* isolates were incubated with a mixture of ampicillin, piperacillin and cefotaxime. and subjected to LC-MS/MS analysis on a SCIEX 3200 QTRAP® hybrid triple quadrupole / linear ion trap mass spectrometer. Panel A demonstrates the detection of ampicillin and piperacillin parent drugs in a sensitive strain with no hydrolysis of antibiotics. Panel B demonstrates a resistant strain which hydrolyses both drugs with the appearance of hydrolyzed product (ampicillin hydrolysis in red, piperacillin hydrolysis product in grey). Panel C demonstrates cefotaxime parent drug in the absence of hydrolysis and Panel D the disappearance of cefotaxime parent drug in the presence of an *E. coli* expressing an extended spectrum beta-lactamase.