Rapid identification and resistance assessment:
The future is mass spectrometry

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Outline

- Introduction
- Plug and play
- Pre-prep and post analysis
- Other wonderful approaches
- Other organisms
- Closing statement
Introduction

- **MS is broad spectrum** – not restricted to pre-specified targets
- **Rapid**
- **Masses of intact molecular ions and fragments easily determined by various types of MS**
- **Tandem MS can produce fragment ions which provide information about molecular structures and sequences**
- **Proteomics determines gene expression** – does not focus on only a single gene
- **Protein expression may vary** – but gene detection may not represent protein expression at all
MALDI-TOF MS

1. Unknown microorganism agar plate sample
2. Smear microorganism directly on target plate
3. Overlay with CHCA matrix solution
4. Generate MALDI-TOF profile spectrum
5. Reference database pattern matching
6. Identified species
MS and Tandem MS (MS/MS)
"THE COMPUTER SAYS I NEED TO UPGRADE MY BRAIN TO BE COMPATIBLE WITH ITS NEW SOFTWARE."
Plug and play

- MRSA – variable reports
- VRE – routine use for VanB, fully automated
- Beta-lactamases
  - *B. fragilis* cfiA positive (MBL) vs cfiA negative
  - Ampicillin resistant *E. coli* – poor reproducibility
  - Clonal differentiation of resistant and susceptible clones cannot be expected for the majority of bacteria
Preparation and analysis required: Detection of enzymatic activity

**Beta-lactamases**
- Variety – difficult to propose universal primers
- MS detection of antimicrobial and degradation product
- Antimicrobial and organism+antimicrobial
  - Spectrum represents beta-lactam molecule, its salts or degradation products is analyzed
  - Detect loss of molecule peaks and presence of hydrolysis products after couple of hours
  - Described for ampicillin, piperacillin, cefotaxime, ceftazidime, ertapenem, imipenem and meropenem
Ampicillin after (A) incubation with the β-lactamase-negative *E. coli* strain DH5α (B) a β-lactamase-producing strain (C) Inhibition of hydrolysis by a β-lactamase-producing strains was performed in the presence of clavulanic acid.

Nonhydrolyzed form of ampicillin peaks are highlighted in gray. Hydrolyzed form of ampicillin peaks are indicated with a small black arrow.
Detection of enzymatic activity

Carbapenem resistance

- Phenotypic and genotypic testing issues with TAT, cost, specificity

- MS – rapid, easy, readily available reagents

  - *Enterobacteriaceae, P. aeruginosa* and *B. fragilis* – incubated with known MBLs and carbapenemases

  - 67/70 carbapenemase producers correctly detected

- Imipenem and degradation products

- Large proportion OXA type carbapenemases in *A.baumannii*
Ertapenem degradation

Ertapenem

+ NDM-1-carrying
K. pneumoniae

+ IMP-1-carrying
P. aeruginosa
Detection of enzymatic activity

- Carbapenem resistance
  - Hydrolysis of ertapenem by *K. pneumoniae* directly from positive blood cultures

- Standardization of *Acinetobacter* carbapenemase detection (May 2013)
  - Comparative analysis of MBL and OXA type producing *Acinetobacter* strains resulted in reduction of imipenem peaks
  - Different effects was observed in different OXA type producers
MALDI-TOF MS spectra showing the most representative imipenem peaks

(A) Incubation solution only
(B) Imipenem-cilastatin solution
(C) OXA-24 like producing Acinetobacter added
(D) IMP-8 Acinetobacter added
Representative comparison of the average intensities of the most representative peaks of imipenem (m/z 300 and 489) obtained after a 1-h incubation with the different carbapenemases included in the study.
Detection of enzymatic activity - conclusions

All studies similar results – this methodology has great potential to become routine

Principle of degradation product monitoring can be applied to other enzymatic resistance mechanisms

However, manual measurement and raw spectra analysis

For ertapenem and *K.pneumoniae* in positive blood cultures – MS calibrated to detect mass accuracy less than 0.5 Da

Interpretation and evaluation – expert knowledge required

After peak assignment of sensitivity and resistance patterns – classification becomes easy

Special software and automation of acquisition and interpretation of results should be investigated and made available

Not able to detect other carbapenem resistance mechanisms. These would include porin alterations and efflux mechanisms for *K. pneumoniae, P. aeruginosa* and porin alterations, efflux mechanisms and PBP alterations for *A. baumannii*. 
Preparation and analysis required

Other

- **rRNA methyltransferase detection**
  - Purified ribosomes and purified enzymes
  - Needs to be simplified to become routine

- **Porin detection**
  - OmpK36 porin in *K. pneumoniae* (responsible for penetration of carbapenems into periplasm)
  - Failure to express OmpK36 due to an insertional inactivation or a nonsense mutation in the *ompK36* gene
  - Specific extraction followed by MALDI-TOF MS
  - Porins detected on molecular weight
Carbapenem-resistant *K. oxytoca* ZC101; and *K. pneumoniae* Z5 and Z4. The arrows with dotted lines indicate the loss of 19,000- and 38,000-\(m/z\) peaks representing OmpK36.
Other approaches

Proteomic approaches

- Entire protein complement expressed is studied
- Selective lysis and electrophoresis
- MALDI-TOF MS after SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis)
  - Described for outer membrane and periplasmic proteins altered in resistant isolates
- not routine
Other approaches

- **Tandem MS**
  - Kanamycin phosphorylation detection

- **Minisequencing**
  - DNA amplification, then allele-specific reactions measured by MALDI-TOF MS
  - Specialized
Other approaches

**PCR-ESI MS**
- Measures m/z of PCR amplicons
- Described for antimicrobial resistance detection
- Detection of mixed bacteria and antimicrobial resistance detection eg. *mecA* or *vanA/B* (specific primers used) in IE

**Nanoparticles**
- MALDI-TOF MS not suitable for detection of small molecules due to matrix interference
- Nanoparticles for example magnetic nanoparticles can enrich and enhance detection of small molecules
- Improves signal to noise ratio
Other organisms

**Antifungal susceptibility**
- *C. albicans* and fluconazole
  - Proteome monitored of *Candida* cells grown with and without antifungal
- *C. albicans* and caspofungin
  - CCI on standard Bruker Microflex used

**Antiviral susceptibility**
- RFMP assay based on MS analysis of small DNA fragments that include sites of mutation
- Distinct peaks relevant to each codon
- NRTIs, NNRTIs and PIs for HIV drug-resistance
- Fast, cost effective
- Bruker Biflex
Summary

MALDI-TOF MS
- Great potential
- Direct detection of antimicrobial resistance (plug and play) already in use
- Enzyme degradation of antimicrobials already in routine use
- Develop more methods
- Better software and automation

Other approaches
- Being investigated
  - Other resistance mechanisms
  - Other MS and technology
  - Different organisms
Proteome-level studies detect behavior of strains
  - Expression of proteins of interest
  - Posttranslational modifications

Most routinely used molecular genetics are restricted to detection of known resistance determinants
  - Including WGS databases

Some proteomic analysis labor-intensive – reference centers

MALDI-TOF MS opens new avenues for clinical and experimental microbiology
References


References


