NATIONAL INSTITUTE
OF INFECTIOUS DISEASES
"Prof. Dr. MATEI BALŞ"

# Broad range PCR coupled with electrospray ionization mass spectrometry – usefulness in a tertiary infectious disease centre

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# Background

- Rapid and reliable diagnosis is needed for timely initiation of appropriate antibiotic treatment
- Novel methods are being developed to overcome the limitations of current culture based methods
- Broad range PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) is a culture-independent method able to detect a broad spectrum of bacteria directly from clinical specimens in less than 8 hours

# Background

PCR/ESI-MS

- PCR primers target broadly conserved bacterial and *Candida* genes
- ESI-MS able to discriminate amplicons
- analysis of base composition signatures of multi-locus amplicons from one or more different species
- detection and identification of more than 670 bacteria and 4 antibiotic resistance markers (*mecA*, *vanA*, *vanB*, $bla_{KPC}$)

Metzgar D et al. (2016) PLoS ONE 11(7):e0158186

# Objective

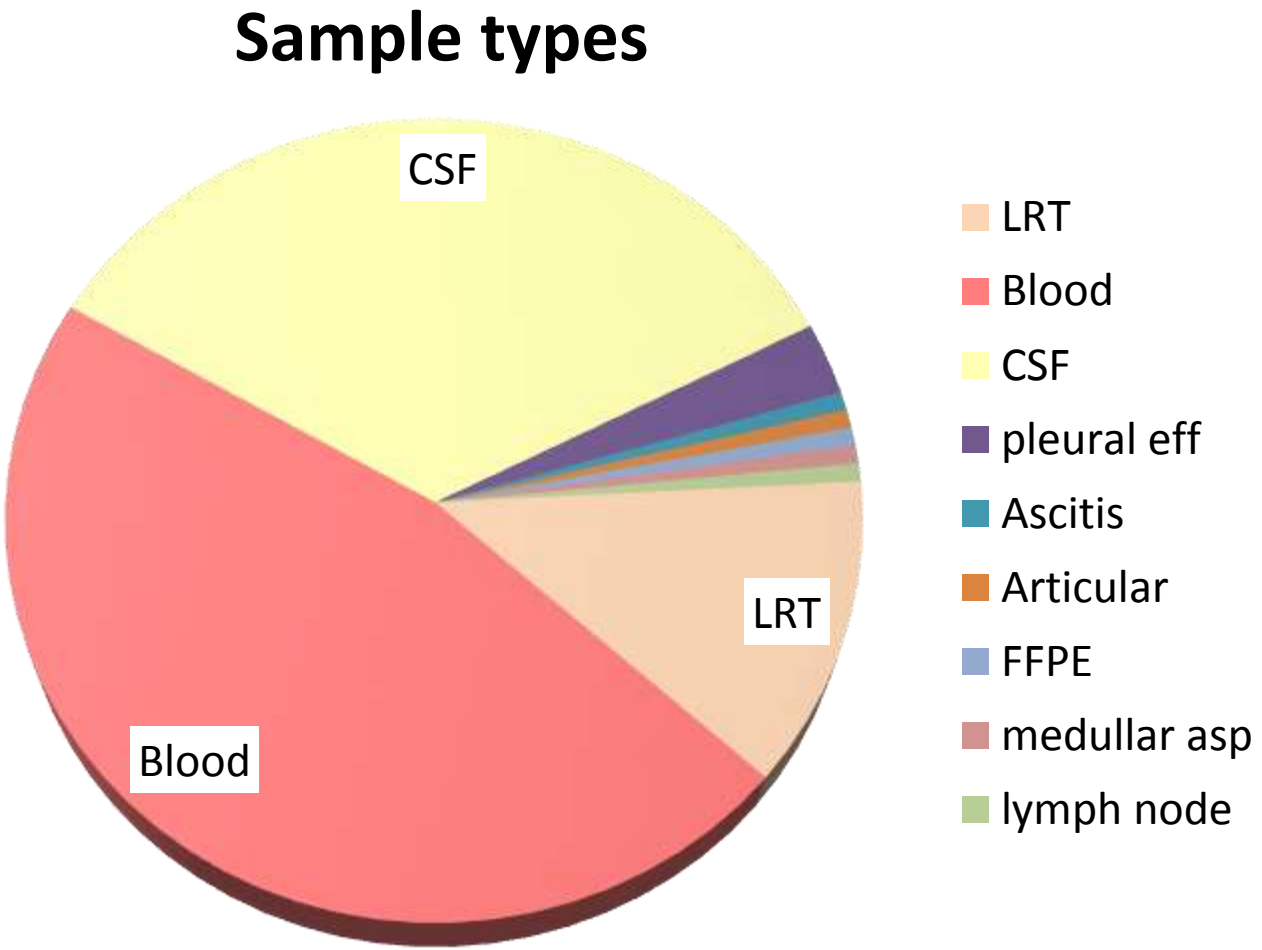
- to evaluate the performance of PCR-ESI/MS compared to the phenotypic methods currently used for the diagnosis of bacterial infections in our tertiary level infectious diseases centre

# Methods

- observational study
- the National Institute for Infectious Diseases "Prof. Dr. M. Bals“ (Bucharest, Romania)
- paired samples collected between March to November 2016 from patients with a clinical suspicion of infection
- standard microbiological phenotypic tests
- PCR/ESI-MS - the IRIDICA System and the BAC Assay kits (Abbott Molecular, Des Plaines, IL) for bloodstream infections (BSI), lower respiratory tract (LRT) and sterile fluids/tissue (SFT)

# Results

- 136 paired samples



# Results

Comparative results of BAC Assays and standard-of-care culture

| Results | No of samples |
|---|---|
| PCR/ESI-MS +, culture + | 24 |
| PCR/ESI-MS +, culture - | 30 |
| PCR/ESI-MS -, culture + | 0 |
| PCR/ESI-MS -, culture - | 82 |

Positivity rate: 39.7% for PCR/ESI-MS and 17.6% for standard microbiological tests

# Results

- Comparative positive results in blood specimens

| Organism | Matched positive | PCR/MS +, culture - |
|---|---|---|
| *Acinetobacter baumannii* | 0 | 1 |
| *Bartonella quintana* | 0 | 1 |
| *Enterobacter cloacae* | 0 | 1 |
| ***Escherichia coli*** | **2** | 3 |
| *Fusobacterium nucleatum* | 0 | 1 |
| *Mycobacterium avium complex* | 0 | 2 |
| *Mycobacterium tuberculosis complex* | 0 | 1 |
| *Neisseria meningitides* | 0 | 1 |
| ***Pseudomonas aeruginosa*** | **1** | 0 |
| ***Staphylococcus aureus*** | **1** | 2 |
| *Staphylococcus epidermidis* | 0 | 1 |
| *Streptococcus pneumoniae* | **1** | 0 |
| *Streptococcus pyogenes* | 0 | 1 |

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| *Fusobacterium nucleatum* | 0 | 1 |
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| *Mycobacterium tuberculosis complex* | 0 | 1 |
| ***Neisseria meningitidis*** | **0** | **1** |
| *Pseudomonas aeruginosa* | 1 | 0 |
| ***Staphylococcus aureus*** | **1** | **2** |
| ***Staphylococcus epidermidis*** | **0** | **1** |
| *Streptococcus pneumoniae* | 1 | 0 |
| *Streptococcus pyogenes* | 0 | 1 |

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| *Staphylococcus aureus* | 1 | 2 |
| *Staphylococcus epidermidis* | 0 | 1 |
| *Streptococcus pneumoniae* | 1 | 0 |
| ***Streptococcus pyogenes*** | **0** | **1** |

# Results

- Comparative positive results in sterile fluids/tissue

| Organism | Matched positive | PCR/MS +, culture - |
|---|---|---|
| *Enterococcus faecalis* | **0** | **1** |
| *Fusobacterium nucleatum* | **0** | **2** |
| *Haemophilus influenzae* | **0** | **1** |
| *Listeria monocytogenes* | **0** | **2** |
| *Neisseria meningitidis* | **0** | **2** |
| *Propionibacterium acnes* | **0** | **1** |
| *Pseudomonas aeruginosa* | **0** | **1** |
| *Serratia marcescens* | **0** | **1** |
| *Staphylococcus aureus* | **1** | **0** |
| *Streptococcus intermedius* | **0** | **1** |
| *Streptococcus pneumoniae* | **0** | **2** |

# Results

- Comparative positive results in respiratory samples

| Organism | Matched positive | PCR/MS +, culture - |
|---|---|---|
| *Acinetobacter baumannii* | **5** | **1** |
| *Coxiella burnettii* | **0** | **1** |
| *Enterococcus faecalis* | **0** | **1** |
| *Enterococcus faecium* | **0** | **1** |
| *Klebsiella pneumoniae* | **0** | **1** |
| *Moraxella catarrhalis* | **0** | **1** |
| *Providencia* | **0** | **0** |
| *Pseudomonas aeruginosa* | **2** | **0** |
| *Staphylococcus aureus* | **2** | **0** |
| *Streptococcus pneumoniae* | **1** | **2** |
| *Ureaplasma urealyticum* | **0** | **1** |

# Results

- Comparative analysis of discordant positive results

| Sample type | Matched positive | Additional PCR/MS detection | Additional culture detection |
|---|---|---|---|
| Blood | *E. coli* | *A. baumannii* | - |
| ETA | *S. aureus* | *M. catarrhalis* | - |
| ETA | *A. baumannii* | - | *Providencia* |
| ETA | *A. baumannii* | - | *E. faecalis* |
| Pleural effusion | - | *F. nucleatum* | *S. anginosus* |
| ETA | *Acinetobacter* | *A. calcoaceticus* | *A. baumannii* |

# Results

- Antibiotic resistance markers detections

| Antibiotic resistance marker | Matched positive | Discordant result | Matched negative |
|---|---|---|---|
| *mecA* | 2 | 0 | 2 |
| *vanA* | 0 | 0 | 0 |
| *vanB* | 0 | 0 | 0 |
| KPC | 0 | 0 | 0 |

# Conclusions

- PCR/ ESI – MS allowed **twice more detections** of a **broader range** of bacteria from various clinical samples in a **shorter time** compared to conventional phenotypic methods.
- This method might have an important impact on timely selection of targeted antimicrobial therapy and avoidance of unnecessary treatments, especially in critically ill patients.
- Further studies of compared performance might help to fully understand the significance of the rather small number of discordant results

Thank you