Development of diagnostic kits for selected markers of resistance, virulence and zoonotic transmission among methicillin-resistant *Staphylococcus aureus* strains

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   b) NALFIA

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   3. Triplex CC398, *mecA* and *cyt b* Real-Time PCR

3. **Outlook**
Q-Bioanalytic GmbH

Existing portfolio

More than 20 Real-Time PCR kits for food safety and quality testing

Selection of Real-Time PCR Kits

1. QuickBlue MRSA
2. OneCup Salmonella
3. OneCup Listeria spec.
4. QuickBlue Listeria monocytogenes
5. QuickBlue Staphylococcus aureus
6. QuickBlue Cronobacter sakazakii
7. QuickBlue Clostridium perfringens
8. QuickBlue E. coli
9. QuickBlue Campylobacter jejuni
10. QuickBlue E. coli, EHEC, EPEC, EIEC, Shigella
11. QuickBlue EHEC (stx1, stx2, eaeA)
12. QuickBlue Vibrio vulnificus
13. QuickBlue Vibrio parahaemolyticus
14. QuickBlue Vibrio cholerae
15. QuickBlue Vibrio alginolyticus
16. QuickBlue Legionella pneumophila
17. QuickBlue Legionella spec.
18. QuickBlue Pseudomonas aeruginosa
Q-Bioanalytic GmbH
Existing portfolio in medical microbiology

- IVD CE marked Real-Time PCR for MRSA
- DNA Purification kit based on magnetic nano-particles
Q-Bioanalytic GmbH

Tasks:

• Development of PCR-based test kits using NALFIA and Real-Time PCR for rapid multianalyte diagnosis of resistance determinants

  a) meca and new mecc homologues
  b) Differentiate between human and livestock-related MRSA lineages such as CC398
  c) Typical relevant virulence or resistance markers
NALFIA

„Nucleic acid lateral flow immuno-assay (NALFIA)“ = Combining molecular biological principle of detection with immunochemical principle of visualization
NALFIA

1. primer one → tag one
   → tag two → primer two
   → amplification

2. double strand double tagged amplicon
   → carbon nanoparticle
   → neutravidin
   → biotin (tag 1)

3. tag two specific anti-tag body
   → membrane

4. result frame

5. absorption pad
Why NALFIA?

1. Bringing PCR to environments outside of labs and to Point-of-Care applications
2. Bringing the analytical knowledge to Point-of-Care users through smartphone applications
Developments

1. NALFIA test addressing \textit{mecA}, \textit{mecC} and \textit{cyt b}

Detection of \textit{mecA}, \textit{mecC} gene and \textit{cyt b} as internal amplification control (IAC) using NALFIA.

Detection limit:
- SCC\textit{mec} VI (\textit{mecA}) 1.5 pg, 10-100 cfu
- SCC\textit{mec} XI (\textit{mecC}), 15 pg, 100-1000 cfu

Seidel et al. "Development of a nucleic acid lateral flow immunoassay (NALFIA) for reliable, simple and rapid detection of the methicillin resistance genes \textit{mecA} and \textit{mecC}.” \textit{Veterinary Microbiology} (2016).
DNA purification and optimization of sampling

Risk assessment concerning limit of detection in clinical samples revealed:

• Insufficient DNA purification can lead to false negative results
• Sampling with certain swabs can lead to insufficient release of the material into the purification reagents
• Time of analysis is a critical factor for acceptance of the methods in clinical settings

Conclusion:
• DNA extraction and/or sampling have to be optimized
Developments
Performance of prior existing DNA purification methods

QuickBlue DNA extraction kit (QBA):

Extraction using spin column technology:

LoD: 300 cfu

3 x 10^4 cfu
Developments

2. New optimized DNA purification procedure

Step 1
Swab sample transferred into a tube with lysis buffer (thermal lysis)

Step 2
Supernatant transferred to tube with binding buffer and silica-magnetite nanoparticles

Step 3
Nano-particles are magnetically immobilized and re-suspended in 100μL elution buffer

Purification time max 30 min
Developments:
2. New DNA purification

Optimized QuickBlue DNA extraction kit (QBA)
Optimization of sampling
Comparison of detection efficiency by Real-Time PCR

Classiq Swabs by COPAN: Tip wrapped with traditional Polyester fiber.

Flocked Swabs by COPAN: FLOQSwabs
Optimization of sampling,

Testing by dipping swabs in a decadal dilution series. A MRSA strain was grown 48hrs in Giolitti broth. Subsequently the culture was diluted and swabs were dipped into it in the presence and absence of 30µl blood.

Classiq Swabs by COPAN: Dipping 30 sec. in each dilution and drying for 5 min.  
Flocked Swabs by COPAN:
Optimization of sampling

Classiq® Swabs by COPAN

FLOQSwabs® by COPAN

Results of experiments in the presence of blood
Results optimization of DNA purification and sampling

1. Risk of false negative results due to insufficient DNA purification could be reduced by reducing the limit of detection from 300 cfu to 15-150 cfu (30 minutes purification time)
2. Recovery rates from swabs in the presence of blood could be enhanced by an order of magnitude through application of FLOQSwabs
Developments
3. Triplex CC398, mecA and cyt b Real-Time PCR

• Specific primers for LA-MRSA CC398 after Stegger et al. (2011)
• Primers for the detection of mecA and cyt b
• Real-Time PCR with SybrGreen®
• Real-Time PCR with TaqMan® probe
Developments

3. Triplex CC398, mecA and cyt b Real-Time PCR

- Pure culture-
  Sample NC PC

- with matrix -
  Sample NC PC

CC398, 296 bp
mecA, 171 bp
cyt b, 123 bp
Developments
3. CC398 Real-Time PCR

Detection of cc398 in HEX channel
Detection of mecA in Cy5 channel
Detection of cyt b in FAM channel

\[ \Rightarrow 0.460 \]
\[ \Rightarrow 4.138 \]
\[ \Rightarrow 0.977 \]
3. Summary

Developments:

a) Multiplex test for *mecA*, *mecC* gene and cyt *b* (IAC) using NALFIA

b) Optimization of magnetic nano-particle-based purification method in combination with FLOQSwabs

c) Triplex test cc398 with Real-Time PCR including internal control

Outlook:

Validation of the CC398 Real-Time PCR
4. Outlook

• Optimization work of the triplex Real-Time PCR system for detection of cc398, mecA, and cyt b

• Validation of the Real-Time PCR for detection of CC398:
  • Sensitivity
  • Specificity
  • Limit of detection
  • Robustness

• Development of NALFIAs to detect:
  • mecA/C, S. aureus and IAC
  • other MRSA resistance genes
  • MRSA virulence genes
Vielen Dank für Ihre Aufmerksamkeit!

Thanks for your co-operation