2021 Best Research Poster Award

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Development of an in house multiplex PCR for identification of *Staphylococcus aureus* resistance genes on clinical isolates

Maxwell Braddick; Michelle Sam; Mythili Tadepalli; John Stenos; Eugene Athan; Stephen Graves; Owen Harris.

INTRODUCTION

Rapid identification of *Staphylococcus aureus* from clinical isolates can shorten the time to appropriate antibiotics[1].Conventional culture and antimicrobial susceptibility testing can take days to return results resulting in the use of broad spectrum empiric antibiotics and delays to targeted management. Identification of resistance genes by PCR can predict phenotypes and avoid the use of unnecessarily broad empiric therapy.

We present a single-centre verification of an in-house multiplex PCR for diagnosis of *S. aureus*.

OBJECTIVES

- Develop a PCR assay for the detection of *Staphylococcus aureus*
- Detect common resistance genes in *Staphylococcus aureus*
- Compare standard phenotypic methods with genotypic resistance profiles

METHOD

149 retrospective *S. aureus* isolates were collected and revived on horse blood agar. Colonies were suspended in solution and bacterial DNA was extracted using a commercial DNA extraction kit. Two parallel multiplex rtPCR assays were performed for the target genes *SCCmec-orfX* and *nuc*, and *mecA* and *blaZ* respectively. An in house-reaction master mix was used.

The target genes were used to label isolates with the following resistance genotypes:

- penicillin susceptible *S. aureus* (PSSA),
- methicillin susceptible *S. aureus* (MSSA)
- methicillin resistant *S. aureus* (MRSA)

The resistance genotypes were then compared with routine phenotypic testing (CLSI method) for concordance.

RESULTS

145 unique isolates were run through the in house PCR

- 20 isolates were identified as **MRSA**
- 93 isolates were identified as **MSSA**
- 32 isolates were identified as **PSSA**

14 isolates were genotype/phenotype discordant

- 2 had a MSSA genotype but PSSA phenotype
- 10 had a PSSA genotype but MSSA phenotype
- 2 had a MSSA genotype but MRSA phenotype

Repeating phenotypic on the revived isolates resolved the discordances

DISCUSSION

Rapid identification of *Staphylococcus aureus* from blood culture isolates is sought after, as evidenced by the availability of an array of commercial PCR kits for various targets[2].

This study shows preliminary feasibility of an assay reliant on existing lab infrastructure rather than commercial kits.

An unexpected outcome of the study was highlighting the variability in local laboratory methods for phenotypically differentiating PSSA and MSSA as evidenced by the 12 discordant results resolved by re-testing with a standardised method (CLSI disc diffusion).

CONCLUSION

This method was successful in identifying *S. aureus* with excellent concordance to phenotypic testing. Initial discordance may be attributable to variable phenotypic testing methods or gene knockout from revived samples . Optimisation of the assay in a prospective study of clinical isolates is underway.

REFERENCES & ACKNOWLEDGEMENTS

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