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Improved Laboratory Reporting Times And Clinical Impact For Panton-Valentine Leukocidin Testing Using The Progenie Realcycler SAPV Real-time P.C.R. Assay.

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Introduction

Staphylococcus aureus can produce a toxin called Panton-Valentine Leukocidin (P.V.L). This can cause severe necrotising infection, leading to severe pneumonia, skin infections and even death[1]. It can spread from person to person and all patients with suspected or known P.V.L. producing *S. aureus* must be isolated in a side room and may be started on additional antibiotics which act against the toxin[3].

Impact on reporting times for the detection of Panton–Valentine Leukocidin genes in *S. aureus* using the Progenie Realcycler SAPV realtime molecular assay, when compared to testing provided at a reference centre, was analysed.

Clinical impact with regards to time in isolation and use of appropriate antibiotics was also evaluated.

Method

During the period 1st August until 31st October 2013, all isolates of *S. aureus* requiring testing for P.V.L. toxin genes were tested using the Progenie Realcycler SAPV real-time P.C.R. assay.

Isolates were subcultured to Columbia Horse Blood Agar (Biomerieux, U.K) and incubated in air at 37°C for 18-24 hours at City Hospital (Birmingham, UK).

4-5 colonies were harvested using a disposable 1µL loop (Sarstedt, U.K.) (Figure 1.) and emulsified in 0.5mL of molecular grade water (VWR, UK) in an 2mL Eppendorf (Figure 2).

The suspension was then heated at 97°C for 30 minutes in order to lyse the bacterial cells and release genomic D.N.A (Figure 3), allowed to cool then centrifuged at 13,000g for 10 minutes to pellet cellular material.

7.5µL of supernatant was added to a Realcycler SAPV assay tube (Instrumentation Laboratory, U.K) (Figure 4).

Method

The assay tube was placed into a Smartcycler (Cepheid, USA) real-time P.C.R. system (Figure 5) and the manufacturers provided assay protocol was run and results analysed[2] (Figure 6).

Figure 1.

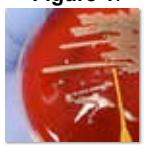


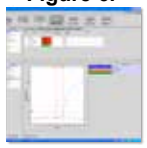
Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.



The average, median and range time to result was recorded.

These were compared to reporting times for isolates tested at a reference centre (PHE Colindale, London UK) and were retrospectively analysed to record time from request for testing for P.V.L. genes to the time the result was reported from the reference laboratory during the period, 1st September-2012 to 31st July 2013.

Review of laboratory clinical notes for all cases was undertaken to establish if the reduced turnaround time impacted on patient care

Results

Impact on Laboratory Turnaround Time

Results provided by reference centre (n=52)
Mean time to result from request = 7.67 days
Median time to result from request = 7 days
Range = 4-15 days

Results obtained using In-House Progenie Realcycler SAPV Assay (n=36)
Mean time to result from request = 1.9 days
Median time to result from request = 1 day
Range = 1-8 days

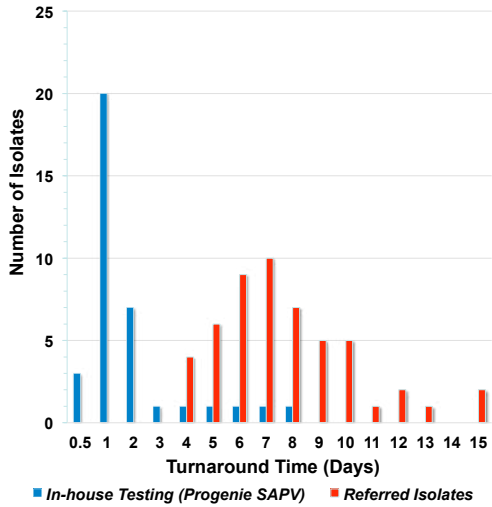
Results

Impact on Laboratory Turnaround Time

When compared, the in-house use of the Realcycler SAPV assay clearly shows an improvement of laboratory turnaround time from 7.67 days to 1.9 days with most available within 1 working day (Figure 7).

Figure 7.

Turnaround Time For P.V.L. Results.



Clinical impact

Review of the 19 patients tested for P.V.L. from samples other than blood cultures showed that all 19 patients had a clinical picture compatible with possible PVL producing *S. aureus*.
Of the 15 patients with blood cultures tested for P.V.L., 14 were septic at the time that the blood culture was taken. 4 patients required intensive care.
One patient, who was P.V.L. positive from a blood culture, was an unexpected case, as the result was from a bacteraemia in a patient with an infected peripheral cannula.
We would not routinely test for P.V.L. in *S. aureus* isolates obtained from an infected cannula site.

Results

Clinical impact

The patient was isolated and appropriate antibiotics commenced within 24 hours of detection.

It is this laboratories policy to test all *S. aureus* bacteraemia isolates for P.V.L.. These results show that it may be difficult to determine if a patient with a *S. aureus* bacteraemia is also positive for P.V.L. toxin, based on clinical grounds alone.

Non-bacteraemia P.V.L. cases identified resulted in a screening programme for two families, potentially reducing transmission within a school in both cases.

In all cases where a negative result was obtained, the antibiotic therapy was reviewed and patients removed from isolation if appropriate.

Conclusions

The Realcycler SAPV assay is simple and easy to use.

Using the RealCycler P.V.L. toxin realtime P.C.R. method has dramatically improved turn-around times for this test.

Having the P.V.L. toxin status on average up to 5 days earlier, tailors the management of these patients in a timely manner and reduces inappropriate antimicrobial therapy and allows better management of isolation resources and prevention of transmission.

References

1. Gillet Y et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 2002; 359:753-9
2. Progenie Realcycler SAPV assay technical documentation version 3, 2013
3. Nathwani D, et al. on behalf of the British Society for Antimicrobial Chemotherapy Working Party on community-onset MRSA Infections. Guidelines for UK practice for the diagnosis and management of methicillin-resistant *Staphylococcus aureus* (MRSA) infections presenting in the community. *J Antimicrob Chemother* 2008;61: 976-994.

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