A simple and rapid detection system for direct identification of carbapenemase-producing Enterobacteriaceae in clinical samples

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1. Abstract

Background: Carbapenemase-producing Enterobacteriaceae (CPE) is a global health issue due to their hostile dissemination through the transfer of carbapenemases genes. Hence, rapid detection is necessary to take relevant control measures against CPE infection/colonization. We established a rapid and multiplex CPE detection system – single-strand tag hybridization – printed array strip (STH-PAS) by targeting the four major carbapenemases. STH-PAS is a DNA-DNA hybridization technique where the oligonucleotide tag in the primer of PCR product hybridizes to its probe imprinted on a chromatographic strip without denaturation. Further, the efficacy of STH-PAS in detecting CPE directly in clinical samples is evaluated.

Methods: STH-PAS was tailored to detect various alleles of the four carbapenemase genes – NDM, KPC, IMP, and OXA-48 like in a single reaction. Then, the efficiency of hybridization in STH-PAS for detection of carbapenemases was compared with conventional PCR. The efficiency of carbapenemase detection by STH-PAS was analysed in CPE (n=49) and non-CPE strains (n=10). A total of 134 CPE suspected samples were subjected to STH-PAS to examine its utility for direct clinical samples.

Results: The ideal conditions for hybridization without non-specificity in STH-PAS was determined. STH-PAS was found to be 10 times more sensitive than conventional PCR techniques. It showed both sensitivity and specificity of 100% for carbapenemase detection in bacterial strains (n=59). As it is not affected by any of the inhibitors of clinical samples, STH-PAS showed 90.92% sensitivity and 98.57% specificity in detecting carbapenemase directly in stool samples (n=114).

Conclusion: The results of the current study show that STH-PAS possesses several advantages as a good detection system for CPE. As it is very rapid and simple to interpret the results with naked eye, STH-PAS could be applied in poorly resourced countries. It has been planned to assess the effectiveness of STH-PAS as a surveillance tool in clinical settings to control the transmission of CPE.

2. Carbapenemase-producing Enterobacteriaceae (CPE)

Carbapenemase:
- β-lactam antibiotic
- Drug of the last resort for several multi-drug resistant bacterial infections

CPE:
- Limits the treatment options for life threatening infections
- Immunocompromised patients at high risk of CPE infections
- 50% of bacteraemia patients are known to die
- Exhibit resistance predominantly through carbapenemase enzymes

Carbapenemase:
- Cleaves the β-lactam ring of carbapenem rendering them inactive
- Disseminate hastily through mobile genetic elements like plasmids
- Main reservoir of resistance
- Could be used as a biomarker for CPE detection e.g., NDM, KPC, IMP, OXA-48


- Developed rapid detection system for CPE by targeting the carbapenemases
- PCR based genotypic technique where labelled primers are used for multiplex PCR
- DNA-DNA hybridization without denaturation is employed to detect the target gene
- Rapid – Presence of CPE in the sample can be detected within 2 hours
- Hybridization can be visualized with the naked eye
- Multiplexity – Detects the four major carbapenemase genes in CPE simultaneously in a single reaction

4. PCR Product Detection: STH-PAS Vs Gel Electrophoresis

To compare the sensitivity of C-PAS and agarose gel electrophoresis in detecting the PCR product, different concentrations of BlaC-PAS positive PCR product was prepared by serially diluting from 10^7 nM to 10^0 nM. They were electrophoresed in 2% agarose gel followed by staining with 0.5 mg/mL ethidium bromide for 20 minutes and visualized under the UV illuminator. The same serially diluted PCR products were hybridized to C-PAS with the above mentioned STH-PAS protocol.

- There is an urgent need for a new and rapid simple detection system for surveillance and controlling the CPE transmission

5. Sensitivity and Specificity of STH-PAS for CPE Detection

Clinical isolates were inoculated into 18 broth (0.25 µg/ml, meropenem) and grown overnight at 37°C with shaking. Genomic DNA was isolated from the overnight culture by QIAamp DNA Mini Kit. STH-PAS was performed and compared with Multiplex PCR followed by gel electrophoresis (gold standard).

- STH-PAS showed 100% sensitivity and specificity for CPE detection in different species of clinical isolates

6. Conclusion

- STH-PAS, a new rapid detection system for CPE was established successfully
- STH-PAS is very rapid than conventional methods for CPE detection
- It showed high sensitivity and specificity for direct clinical specimens
- It could serve as a powerful tool for CPE detection in poorly resourced countries
- In the near future, STH-PAS will be employed in hospitals for prospective surveillance

Clinical specimens will be directly subjected to STH-PAS. If they were CPE positive, appropriate patient cohorting and contact precautions will be implemented. Follow-up examination at regular intervals will be performed to check CPE transmission. Ultimately, nosocomial transmission of CPE will be controlled gradually.