# 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 hasty dissemination through the transfer of carbapenemase genes. Hence, rapid detection is necessary to take relevant control measures against CPE infections/colonization. We established a rapid and multiplex CPE detection system – single-strand tag hybridization – printed array strip (STH-PAS) by targeting the four different 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 114 CPE suspected stool samples were subjected to STH-PAS to examine its utilization 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 inhibitory substances in clinical specimens, STH-PAS showed 90.91% 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.

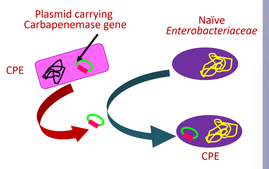

## 2. Carbapenemase-producing *Enterobacteriaceae* (CPE)

**Carbapenem:**
- 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 bacteremia 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

### Spreading Mechanism of CPE




CPE is emerging as an alarming threat in the health sector due to its rapid spreading among the immunocompromised patients. Hence, CPE detection at the earliest is necessary to control their nosocomial transmission. And, hospitals lacking in sophisticated infrastructure need simpler detection systems. In many developing countries, few or no epidemiological data is available on CPE prevalence and their different types of carbapenemases. Hence, it is very difficult to detect the CPE

### Currently available CPE detection systems

Disk diffusion
Broth-microdilution
E-test
Modified Hodge test
Carba NP
CIM
MALDI-TOF
PCR
LAMP
Automated Systems

### Limitations

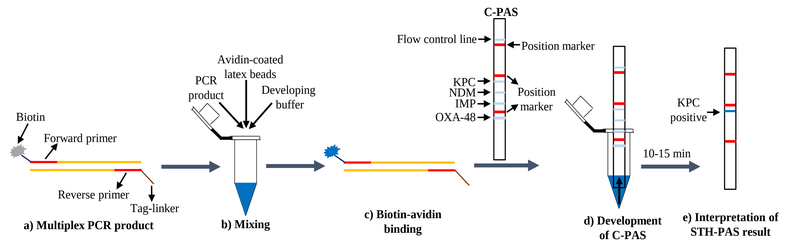
Low specificity
Highly expensive
No Multiplicity
Expertise requirement
Low sensitivity
Not for clinical samples
Time Consuming

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

## 3. Single-strand tag hybridization – printed array strip (STH-PAS)

- Newly 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
- Rapidity – Presence of CPE in the sample can be detected within 2 hours
  – Hybridization can be visualized with the naked eye
- Multiplicity – Detects the four major carbapenemase genes in CPE simultaneously in a single reaction (NDM, KPC, IMP, OXA-48)

### Schematic representation of STH-PAS

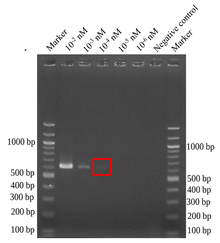



**a)** Initially, multiplex PCR is performed with the forward and reverse primers of each gene labelled with a distinct single stranded tag-linker sequence and biotin (Tohoku Bio-Array), respectively or vice versa. **b)** 5 µl of multiplex PCR product is mixed well together with 5 µl of sterile water, 10 µl of developing buffer and 1 µl of avidin-coated blue latex beads. **c)** If amplification occurred for any of the four carbapenemase genes, the biotin on one primer of the PCR product binds to latex beads during mixing causing the reaction mix to turn blue. **d)** Chromatographic Printed Array Strip (C-PAS) imprinted with the probe for each tag-spacer sequence of the primers at different positions is dipped into the reaction mixture. Through capillary action, the reaction mixture is carried upwards along the C-PAS. **e)** During the capillary motion, the tag-spacer sequence on the other primer of PCR product will hybridize to its respective probe forming a blue colour line which can be visualized with the naked eye within 15 min.

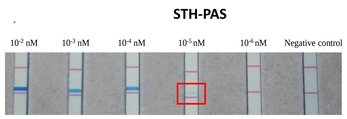
## 4. PCR Product Detection: STH-PAS Vs Gel Electrophoresis

To compare the sensitivity of C-PAS and agarose gel electrophoresis in detecting the PCR product, different concentrations of *bla*$_{IMP}$-positive PCR product was prepared by serially diluting from $10^{-2}$ nM to $10^{-6}$ nM. They were electrophoresed in 2% agarose gel followed by staining with 0.5mg/L ethidium bromide for 20 min and visualized under the UV transilluminator. The same serially diluted PCR products were hybridized to C-PAS with the above mentioned STH-PAS protocol.

**Gel Electrophoresis**




**STH-PAS**



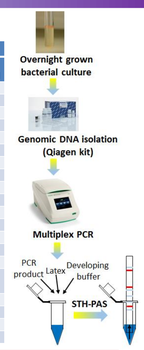

Gel electrophoresis detects $10^{-4}$ nM of PCR product whereas C-PAS hybridization could detect a minimum of $10^{-5}$ nM PCR product which showed that C-PAS is **10 times** more sensitive than electrophoresis in detecting the PCR product

## 5. Sensitivity & Specificity of STH-PAS for CPE Detection

### STH-PAS for genomic DNA of clinical isolates

Clinical isolates were inoculated into LB 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)

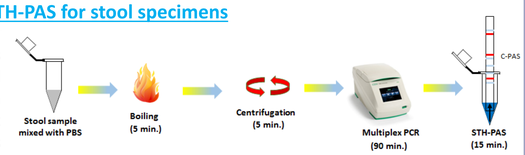
| Isolate type | Carbapenemase gene | Carbapenemase variants | Species | No. of isolates: Multiplex PCR Positive /Total | No. of isolates: STH-PAS Positive/Total |
|---|---|---|---|---|---|
| CPE | $bla_{KPC}$ | KPC-2 | *Klebsiella pneumoniae* | 3/3 | 3/3 |
| | | | *Enterobacter aerogenes* | 3/3 | 3/3 |
| | | | *Enterobacter cloacae* | 2/2 | 2/2 |
| | | | *Escherichia coli* | 2/2 | 2/2 |
| | $bla_{NDM}$ | NDM-1 | *E. coli* | 6/6 | 6/6 |
| | | | *K. pneumoniae* | 5/5 | 5/5 |
| | | | *E. cloacae* | 3/3 | 3/3 |
| | | NDM-5 | *E. coli* | 2/2 | 2/2 |
| | $bla_{IMP}$ | IMP-6 | *E. coli* | 7/7 | 7/7 |
| | | | *K. pneumoniae* | 8/8 | 8/8 |
| | | | *E. cloacae* | 1/1 | 1/1 |
| | $bla_{OXA-48}$ | OXA-48 | *E. coli* | 1/1 | 1/1 |
| | | | *K. pneumoniae* | 2/2 | 2/2 |
| | | OXA-181 | *K. pneumoniae* | 4/4 | 4/4 |
| | | OXA-232 | *K. pneumoniae* | 3/3 | 3/3 |
| | $bla_{NDM}$ & $bla_{OXA-48}$ | NDM-1 & OXA-232 | *K. pneumoniae* | 5/5 | 5/5 |
| Non-CPE | --- | --- | *E. coli* | 0/1 | 0/1 |
| | | | *K. pneumoniae* | 0/3 | 0/3 |
| | | | *E. cloacae* | 0/5 | 0/5 |
| | | | *E. aerogenes* | 0/1 | 0/1 |




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

### STH-PAS for stool specimens

To assess the utility of STH-PAS for direct clinical specimens, stool samples of hospitalized patients were mixed with 1 ml saline and boiled for 5 min followed by centrifugation for 5 min at 13,000 rpm. For 5 µl of supernatant as template, multiplex PCR was performed with the labelled STH-PAS primers followed by STH-PAS as described earlier. The results of STH-PAS were compared with multiplex PCR to determine its sensitivity and specificity for direct clinical specimens



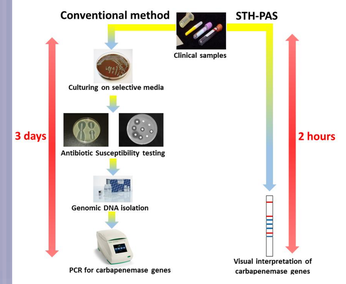

| | | Multiplex PCR: Positive | Multiplex PCR: Negative | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | PLR |
|---|---|---|---|---|---|---|---|---|
| STH-PAS | Positive | 46 | 1 | 92 (80.8-97.8) | 99.4 (96.8-99.9) | 97.8 (88.7-99.9) | 97.7 (94.3-99.4) | 158.2 |
| | Negative | 4 | 171 | | | | | |

STH-PAS showed 92% sensitivity and 99.4% specificity in detecting the CPE in direct clinical specimens

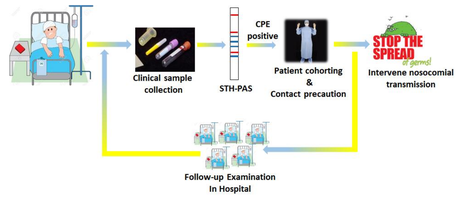
## 6. Conclusion

- ✓ 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

### CPE detection in clinical samples




### Controlling nosocomial transmission of CPE by STH-PAS




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.