Development of a Thermostabilized Cholera-NASBA-ELISA Assay for the Specific Detection of *Vibrio cholerae*

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Cholera is a diarrheal disease caused by *Vibrio cholerae*. It is potentially lethal if not diagnosed early. Conventional culture and biochemical tests for cholera are laborious. Although molecular-based methods are rapid, offer better sensitivity and specificity, they require expensive equipments and cold storage of reagents. Furthermore, DNA-based tests such as PCR do not distinguish between viable and non-viable cells. Nucleic acid sequence-based amplification (NASBA) is an isothermal amplification technique that specifically amplifies RNA, hence detecting viable cells only. In this study, a cholera-NASBA-ELISA assay was developed for detection of *lol*B gene of *V. cholerae* and the feasibility of thermostabilizing the NASBA mix was explored. NASBA conditions were optimized and its amplicons were detected using ELISA. The assay was tested with 41 reference strains comprising *V. cholerae*, *Vibrio* species and enteric pathogens. The clinical evaluation of the assay was carried out using spiked stool samples. The cholera-NASBA mix was thermostabilized by lyophilization and its stability was evaluated at different temperatures periodically. In addition, the ability of the assay to detect only viable cells was investigated by subjecting *V. cholerae* cultures to various lethal treatments and detecting their NASBA signal. The cholera-NASBA-ELISA had an analytical sensitivity of 10 CFU/ml at the bacterial level and 10^2 molecules/μl RNA transcript at the gene level. Diagnostic evaluation with spiked stool samples gave 100% sensitivity, 84.52% specificity, 89.92% positive predictive value and 100% negative predictive value. The thermostabilized NASBA mix was stable for 2 months at 8 °C and −20 °C. In the viability assay, *lol*B mRNA was detected even after 48 hours post-treatment, therefore precluding its use as a viability indicator. Hence, we have for the first time developed a thermostabilized cholera-NASBA kit that reduces multiple pipetting steps and is highly sensitive.

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Epidemiology and Pathogenicity of Methicillin-Resistant *Staphylococcus aureus* Isolates from Pediatric Patients in China

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Background: Our study was to investigate the genetic differentiation and pathogenicity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from the five biggest pediatric hospitals, located in four different regions in China from 2005 to 2006.

Methods: Seventy-three MRSA isolates were analyzed by a combination of different genotyping methods, including multilocus sequence typing (MLST), SCCmec typing and *spa* typing. Additionally, Panton-Valentine Leukocin (PVL) gene was detected. Susceptibility tests were performed for 14 antimicrobial agents. Clinical information about these MRSA isolates was also collected.

Results: Among 73 MRSA isolates, 14 sequence types (STs) of MLST were identified, including two novel types. The SCCmec types of most MRSA strains were type III (42.5%) and type IV (34.2%). Seventy-one strains were differentiated into 19 spa types, including three novel types. Also, 30.1% of MRSA isolates were found to carry the PVL gene. The prevalent strains were ST239-MRSA-III and ST1-MRSA clones in the northern region; ST239-MRSA-III, ST910-MRSA-IV and ST88-MRSA in the eastern region; and ST59-MRSA in the southern region. Only the ST910-MRSA-IV clone (PVL gene-positive) has been found in China until now, and it is closely related to ST30-MRSA-IV. All MRSA isolates were found to be resistant to penicillin and azithromycin, and susceptible to vancomycin. Resistance to other antimicrobial drugs tested was relatively higher and multidrug resistance was also observed. The cases of necrotic pneumonia, severe skin and subcutaneous tissue infection and cervical lymphadenitis resulted from PVL gene-positive MRSA.

Conclusions: A combination of different genotyping methods proved useful for studying the endemic clones of MRSA isolated from children in China. There was a obviously geographical variation in the prevalence of MRSA strains. Antimicrobial susceptibility tests showed high resistance of many antimicrobials and multiple drugs. PVL gene-positive MRSA was likely to be associated with the necrotic process in clinical infections.

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