A rapid two-step algorithm detects and identifies macrolide and β-lactam antibiotics resistance genes

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Background: Antibiotic resistance has rapidly grown into a worldwide problem that threatens to compromise the effective treatment of a plethora of diseases. A two-step test algorithm was developed for detection and identification of macrolide and β-lactam antibiotics resistance genes.

Methods & Materials: Bacterial isolates were first tested by a multiplex real-time PCR (mRT-PCR) covering eight genes in relation to macrolide (msrA, ermA, ermB, and ermC) and β-lactam (blaTEM, blaSHV, blaCTX-M-1, blaCTX-M-9) resistances and the presence of one or multiple resistance genes were determined by melting temperature (Tm) profile analyses. Amplification products with indistinguishable Tm profiles were tested subsequently by a liquid beads microarray assay (LBMA) assay with the eight resistance gene-specific probes. The clinical validity was evaluated on 340 clinical isolates with phenotypic antimicrobial susceptibility results used as the standard comparison methods.

Results: It has been shown that 75% of isolates can be identified by mRT-PCR and melting temperature analyses only, while the rest 25% need both two approaches. An overall agreement of 96.6% (kappa = 0.93, 95% CI = 0.89-0.96) was observed between the mRT-PCR-LBMA and phenotypic methods. The sensitivities and specificities were 94.5% and 97.5% for macrolide and 92.6% and 98.4% for β-lactam resistance determination, respectively.

Conclusion: The two-step mRT-PCR-LBMA assay provided a cost-effective tool for rapid determination of macrolide and β-lactam antibiotic resistances.

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A three year review of antimicrobial resistance of Salmonella enterica serovars Typhi and Paratyphi A in Pakistan

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Background: Enteric fever is among the most common bacteraemic illnesses in South Asia. Multi drug resistance (MDR), defined as resistance to the 3 first line classes of antimicrobial agents chloramphenicol, ampicillin, trimethoprim/sulphamethoxazole has become prevalent in most of South Asia, with a frequency ranging from 50% to 80% of all S.Typhi isolates. Multi-drug resistance as well as fluoroquinolones resistance has severely limited therapeutic options in high disease burden countries such as Pakistan. This review was carried out to determine the frequency of drug resistant Salmonella enterica serovar Typhi (S.Typhi) and Salmonella enterica serovar Paratyphi A (S. Paratyphi) from 2009-2011

Methods & Materials: This was a cross-sectional laboratory based review of records. From January 2009 to December 2011, a total of 116691 blood culture specimens from all age groups were submitted to the clinical laboratory from all over the country. No specific details of history were recorded, only laboratory data was retrieved. Blood specimens were processed in the BACTEC system (Becton and Dickenson, USA). Antimicrobial susceptibility tests were performed using the Kirby Bauer disc diffusion method in accordance with the Clinical and Laboratory Standard Institute (CLSI) guidelines.

Multidrug resistance was considered if isolates were resistant to 3 drugs (Ampicillin, Chloramphenicol and Co-trimoxazole), while Fluoroquinolone resistance was labeled for isolates resistance to either Ofloxacin or Ciprofloxacin.

Results: Sensitivity data of 4323 positive isolates of S. Typhi and S. Paratyphi A, isolated during the three year period were reviewed. The majority of isolates were of S. Typhi (59.6%). Over three years MDR S.Typhi remained high, ranging from 64.8% – 66.0%, while MDR S. Paratyphi A decreased from 4.2% to 0.6%. Fluoroquinolone resistance increased for S.Typhi from 84.7% to 91.7%. Pattern of antimicrobial resistance was uniform across all ages except for two isolates (0.08%) recovered from children aged three years and four years respectively with resistance to third generation cephalosporin.

Conclusion: Our results show high rates of MDR and fluoroquinolone resistance amongst S.Typhi and S. Paratyphi. Occurrence of two cases of ceftriaxone resistance is alarming.

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