



## Molecular Typing of Antibiotic-Resistant Bacteria

**Cheng-Hsun Chiu**

Department of Pediatrics  
Chang Gung Children's Hospital, Chinese Taipei

From an epidemiological point of view, it is often necessary to determine the clonality of the bacterial isolates. This is particularly important in endemic and epidemic nosocomial outbreaks of bacterial infections to improve the management of such outbreaks. Recently, approaches at the molecular level have been used to assess the relatedness of bacterial isolates. Plasmid pattern analysis was found to be useful for the characterization of epidemic strains harboring plasmids. Its discriminatory power is further increased by restriction endonuclease digestion. More recently, sensitive and reproducible molecular markers, including those used in ribotyping<sup>1</sup> and pulsed-field gel electrophoresis (PFGE),<sup>2,3</sup> have been applied with success to many bacterial species. Despite the reliability and broad applicability of these techniques, their use in clinical microbiology laboratories has been limited because they are time-consuming and labor intensive. To circumvent these problems, a number of DNA fingerprinting strategies based on the PCR amplification of variable-length chromosomal sequences with a variety of primers were developed.<sup>4</sup> One of these approaches, known as the random-amplified polymorphic DNA (RAPD) assay, is based on the use of simple arbitrary primers in a PCR of low stringency to amplify segments of the genome and has been used successfully for the typing of several bacterial species. This method has the advantage that no prior sequence information is required, but the fingerprint patterns have a critical dependence on reaction conditions and substrate concentration. On the other hand, a new typing method called infrequent-restriction-site PCR (IRS-PCR) has been proposed by Mazurek GH and colleagues.<sup>5</sup> The main strategy of this method is the selective amplification of DNA sequences located between a frequently occurring restriction site and an infrequently occurring restriction site by using adaptors and primers based respectively on the two enzyme cutting sites.

We recently used two molecular typing methods, PFGE and IRS-PCR, to investigate two clusters of nosocomial bacteremia caused by multidrug-resistant *Klebsiella pneumoniae* in a paediatric intensive care unit.<sup>6</sup> Totally 56 *K. pneumoniae* isolates were analysed in this study. These included 10 bacteraemic isolates from eight patients, 26 isolates obtained during the environmental survey, and 20 epidemiologically non-related isolates incorporated as controls. One major pattern was demonstrated in 22 of the 56 isolates analysed. These included nine of the 10 bacteraemic isolates, the only single rectal isolate, two hand culture isolates, and 10 sink isolates. All of these 22 isolates illustrated identical antibiograms, while the other 34 isolates shared six antibiograms and 31 unique patterns by either PFGE or IRS-PCR assay. The two clusters of bacteremia appeared to be outbreaks induced by the same strain of *K. pneumoniae* which may have utilized the sinks as reservoirs and have been transmitted through the hands of medical personnel to the patients. IRS-PCR is able to demonstrate concordant results with PFGE analysis in studying the genetic relationships among *K. pneumoniae* isolates, and may well serve as an excellent epidemiological tool for this bacterium.

In another example we used these methods to investigate the recent emergence of *Salmonella enterica* serotype Enteritidis infection in Taiwan.<sup>7</sup> Of the 71 clinical strains isolated in 1997-1999, 61 (86%) remained susceptible to the eight antibiotics tested, while the remaining ten, eight of which were isolated in 1999, were resistant to one to three of the agents including three multi-drug resistant strains. PFGE revealed three major genotypes (Types A, B and C), in which Type A was the predominant type. Of the 68 Type A, which contained 8 subtypes, 59 (83%) belonged to only two of the eight subtypes. Similar results were obtained with IRS-PCR. Both two methods detected the types that were rarely seen before and most of these were of recent isolates, indicating that these unusual types were new or strains of foreign origin.

Up to now, we have applied IRS-PCR successfully for typing many medically important microorganisms, including strains of Gram-negative bacilli (*Escherichia coli*, *Enterobacter cloacae*, *K. pneumoniae*, *Serratia marcescens*, *Acinetobacter baumannii*, *Burkholderia cepacia*, non-typhoid *Salmonella*), Gram-positive cocci (staphylococci, streptococci), yeast (*Candida* species), and mycobacteria. Most of these organisms are well known of being resistant to multiple antimicrobial agents. IRS-PCR appears to have a discriminatory power comparable to that of PFGE, but is less tedious and less laborious than PFGE. We think that it can be a potentially universal tool for molecular epidemiological analysis of infections caused by resistant bacteria.

## References

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