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Applications and relevance of plasmid analysis in clinical microbiology laboratories

Multi-resistant enterobacteria are now a well-documented and frequent cause of epidemic nosocomial infections. Notable examples of multi-resistant opportunistic pathogens include *Klebsiella aerogenes* and *Serratia marcescens*. These bacterial species have been consistently implicated as sources of transmissible resistance plasmids which are capable of intraspecific or interspecific spread, so contributing to sequential outbreaks of infection due to different serotypes of the same species (Markowitz et al., 1980; Bidwell, Reeves & Bullock, 1981; Caswell, Talsania & Knight, 1981; Hughes, Datta & Faikers, 1981; Knight & Casewell, 1981) or to different genera (Thomas et al., 1977; Elwell, Inamine & Minshew, 1978; Tompkins, Plorde & Falkow, 1980; Knight & Casewell, 1981; Rubens et al., 1981).

Epidemiological studies of resistant nosocomial isolates may be considered important for two reasons. Firstly, they can provide evidence of intra- or interhospital spread of single multi-resistant bacterial clones, thereby indicating the need for cross-infection control measures. Secondly, epidemiological data may reveal that similar but distinguishable resistant bacterial populations are being selected and maintained in specific wards, units or hospitals. Implementation, or increased stringency, of an anti-
undertaken, may usually be limited to resis-
personnel and equipment. Genetic studies, if
the clinical need and the availability of time,
of plasmid studies must be influenced by
Any decision as to the extent and direction
investigation of antibiotic-resistant enterobacteria.
plasmid components for individual charac-
terization. Plasmid incompatibility grouping
(Datta, 1979), though useful in certain situa-
tions, may not always provide data of
epidemiological significance, since some
plasmids exhibit anomalous compatibility
properties (Grant, Bird & Pittard, 1980) and
many cannot be assigned to recognised
groups. For physical characterisation of plas-
mids in clinical isolates of enterobacteria,
rapid screening methods are the most suitable
for application in routine clinical micro-
biology laboratories. Several rapid methods
have been described for the physical charac-
terization of plasmids by visualisation of
DNA in crude cell lysates (Meyers et al.,
Collective disadvantages inherent in these
methods include requirements for multi-stage
preparation of DNA, phenol or phenol-
chloroform extraction of proteins followed by
a centrifugation step, heat denaturation of
chromosomal DNA fragments, partial purifi-
cation of plasmid DNA, and the appearance
of linear chromosomal, linear plasmid, and
open circular plasmid DNA derivatives,
which may interfere with the visualisation or
identification of superhelical plasmid DNA.
A simple and rapid method recently des-
cribed by Bidwell, Lewis & Reeves (1981)
eliminates each of the above requirements by
the use of a single colony lysate electro-
phoretic technique, and the resulting super-
helical plasmid DNA is selectively visualised
without the appearance of the contaminating
DNA species described above. This method
is particularly suited for application in
routine clinical laboratories, where access to
specialised equipment is not available and
where genetic or molecular biological expert-
tise may be limited.

Other current areas of plasmid research
include restriction endonuclease analysis,
identification and molecular characterisation
of transposons and insertion sequences,
molecular cloning of genes using plasmids,
phages or 'cosmids' as cloning vehicles, DNA
sequencing, and hybridisation of radio-
labelled DNA probes to either DNA pre-
pared 'in situ' from bacterial colonies (colony
hybridisation) or to electrophoretically-
separated endonucleolytic DNA fragments.
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transferred from agarose gels to nitrocellulose paper by 'blotting'. The principles of these molecular techniques are described by Old & Primrose (1980). Although generally beyond both the needs and scope of clinical laboratories, molecular and genetic manipulations of this type are invaluable in the elucidation of plasmid fine structure, function and evolution.

In conclusion, it is both epidemiologically valuable and technically feasible for routine clinical microbiology laboratories to investigate the occurrence and spread of plasmids in their own isolates of antibiotic-resistant enterobacteria. Routine monitoring of such isolates can rapidly provide useful epidemiological data which may indicate the early implementation, and thereby increase the likely success, of cross-infection control measures or suitable antibiotic policies.

J. L. BIDWELL
Department of Microbiology,
Southmead Hospital,
Bristol BS10 5NB.
England

References


