Bacterial Typing Methods
Dr. Giles Edwards
A Webber Training Teleclass – April 8, 2004

Bacterial Typing Methods and Their Value in Infection Control

Giles Edwards
Consultant Microbiologist
Stobhill Hospital, Glasgow, UK
Scottish MRSA Reference Laboratory

Hosted by Paul Webber
paul@webbertraining.com

www.webbertraining.com

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Intention

Illustrate a range of microbial typing methods
- Not concentrating on technical issues ***but***
- On the kind of information obtained & its value in Infection Control (day to day and longer term planning)

Examples from my own experience
- Scotland – only a part of Britain (10% of population)
- MRSA (but not exclusively)

(A personal account from experience in Ref Lab and on Wards)

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Plan of Teleclass

MRSA in Scotland
- History in relation to Britain, Europe and the rest of the world

What infection control practitioners want
- Short term (outbreaks) and long term (surveillance and planning)

Typing methods
- What information typing can provide
- What different methods do provide

Some final comments

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MRSA in Scotland

Situation closer to rest of Britain than to other parts of Europe
- Europe itself having a wide range of experience

Before 1990
- Not a serious problem - interesting to microbiologists ***but***
- Not a serious clinical problem and seemed controllable as IC risk
- Some hospitals monitored some areas
- Typing limited so spread of strains not always recognised

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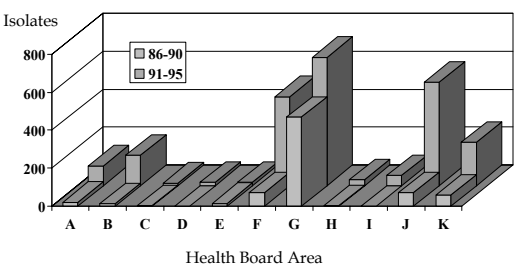
MRSA Isolates Reported to SCIEH
by Hospital Laboratories - 1986-1995



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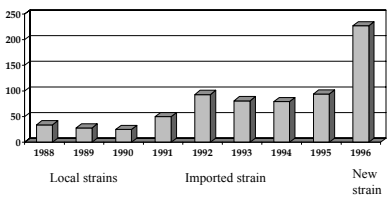
MRSA in Scotland – Early 1990's

More hospitals involved, numbers still quite low, little typing information

Numbers of MRSA Patients in One Hospital



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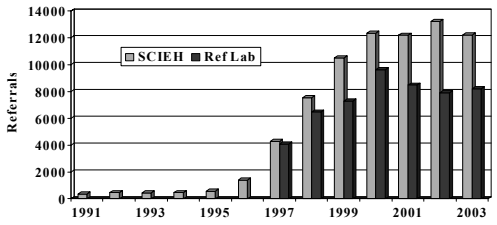
MRSA Reported to SCIEH



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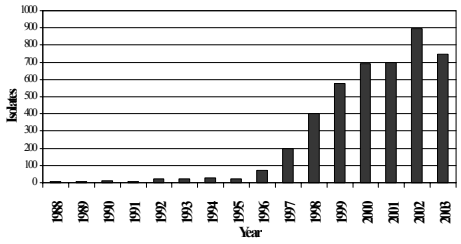
MRSA in Scotland – Blood Culture Isolates (1986 - 2003)



Based on SCIEH Reports

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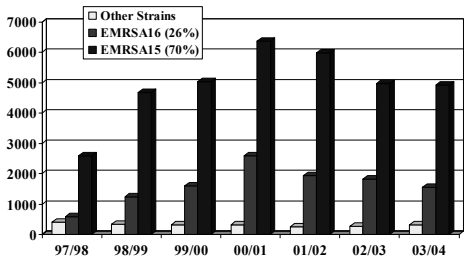
MRSA Typing in Scotland



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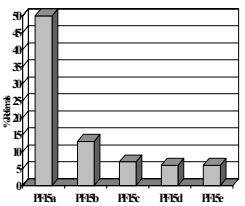
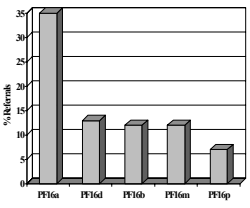
Prevalence of EMRSA15/16 Subtypes



50% EMRSA15 have pattern PF15a

90% EMRSA15 have one of five patterns



35% EMRSA16 have pattern PF16a

79% EMRSA16 have one of five patterns

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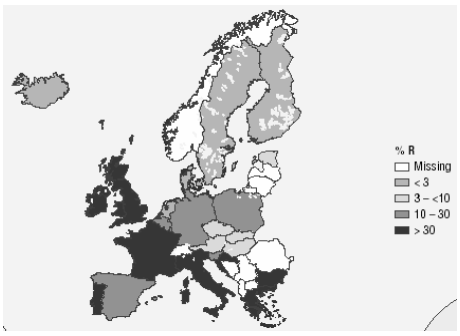
EARSS Data

(European Antimicrobial Resistance Surveillance System)



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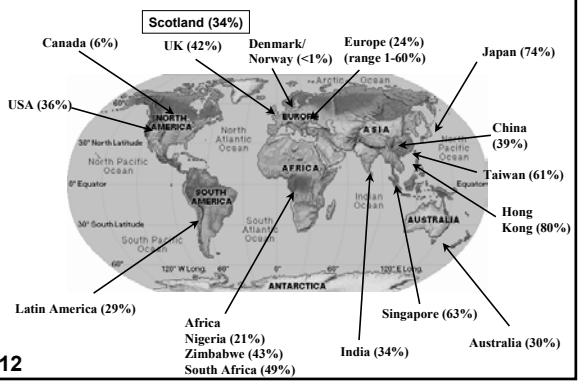
World-Wide Prevalence of MRSA



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What Infection Controllers Want from Typing (1)

Outbreak Investigation

Is this an outbreak?

- Are these isolates part of a group with a recent common ancestor?
- How common are such strains in the background population?
- Is there anything unusual about them (pathogenicity, transmissibility)

How soon can you tell me?

- (I have to do ***something*** today)

Please explain what the results mean.

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What Infection Controllers Want from Typing (2)

Surveillance and Planning

Are changing numbers associated with changing strains?

- Strains with different characteristics
- Pathogenicity, transmissibility

How do these isolates compare with those from other places?

- Are other places better at controlling them

(Please give me the results yesterday)

(Please explain what the results mean)

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What Information can Typing Supply

Similarity between isolates

Wide range of characters that could be examined

- Significance of differences needs to be understood
- Significance of differences needs to be clearly expressed

Relationship to other strains

Comparator strains must

- Be recognisable by the typing methods
- Have known characteristics

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Typing Methods

Key Questions:

- How variable is the characteristic used to compare isolates?
  - Too much variation or too little can both be unhelpful
- How much does the environment contribute to the variation?
- How do results correspond to those of other typing methods?

Too many methods to describe all - but two broad groups:

- Phenotypic – observable characteristic (genes and environment interacting)
  - Under standard conditions may be very close to genetic
- Genotypic – genetic constitution – examination of DNA
  - Closer to the 'recent ancestor question'

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Phenotypic Typing Methods

Widely available for several decades

May be quicker and more readily available (but not always)

Significance usually requires organism specific experience

Examples

- Antibiotic resistance typing – many bacteria
- Phage typing – eg *Staph. aureus*, *Salmonella*
- Serotyping – eg *Salmonella*, *Neisseria meningitidis*, *Legionella*

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Antibiotic Resistance Typing

Can be done in a routine lab – so can be quick

- Some information available anyway by the time an MRSA is recognised
- Many methods but comparable for clinical reasons

Interpretation

- Knowledge of local patterns
- Knowledge of common resistance mechanisms (may be misleading)

Useful preliminary guidance (value often underrated)

- May detect significant difference not picked up by available genotyping
- Local monitoring of continuing outbreak

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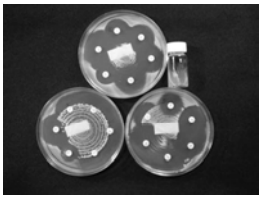
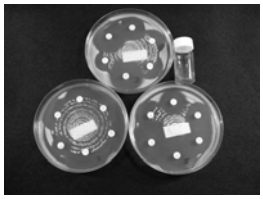
Antibiotic Resistance Typing



Typical EMRSA15



Typical EMRSA16

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Biotyping

Biochemical (usually) eg urease, sugar fermentation

- Some of advantages of antibiotic resistance typing
  - Standardised between labs and widely available
- Less often useful (but urease in British MRSA an exception)
- Combination of single tests developed to identify species rather than subtypes

Interpretation

- Knowledge of local strains

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Serotyping

Well established method – often being superseded by genotyping

Antibodies to variable antigens (often cell wall or cell membrane) prepared and, with a choice of methods (eg latex agglutination, ELISA) used to assign an isolate to a group or type.

Antibodies can be distributed to different labs to allow comparisons

Rapid (same day by many methods) and cheap (once set up)

- Full typing usually incurs delay (getting to Reference Laboratory)

Still used for *Salmonella* 'speciation', also *Legionella*.

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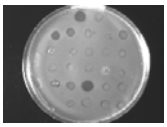
Phage Typing (1)

Most bacteria are susceptible to bacteriophages and susceptibility can fairly easily be shown by lytic plaques on agar plate cultures

Patterns of susceptibility to a carefully selected group of phages gives clear differences between strains (new strains may need a new group)

Difficult to set up and maintain, fairly quick and cheap to run.



EMRSA16 Phage plate

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Phage Typing (2)

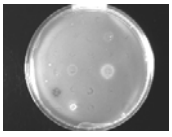
Reliable interpretation needs scrupulous methodology and quality control

Less widely used now but can subdivide common some *Salmonella* serotypes and also *Staph. aureus.*

Designation of Phage Types needs careful consideration -

- Type number or list of phage reactions

83Cw/29ih/52ih/52Aw/79w/80ih/75w/77w/83Aw/94w.



EMRSA15 Phage plate

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Genotypic Typing Methods

More recently developed and often more expensive

Less readily available (at present) and usually slower even if 'on site'

Significance often requires organism specific experience

- but general principles perhaps more easily applied

Examples (chosen from many – "YATM")

- Plasmid profiling
- Restriction enzyme based typing eg Pulsed Field Gel Electrophoresis
- Sequence based methods

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Plasmid Profiling

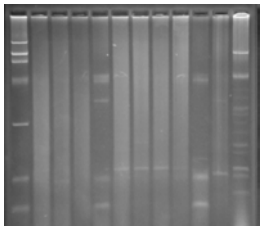
One of the oldest genotypic methods (separating plasmids on gels)

Not all clinically significant bacteria have detectable plasmids

Less stable than many genotypic features

Often relatively quick

May add significantly (for eg salmonellae) to discrimination of PFGE



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PCR based Typing

Many variants

- Use PCR to produce multiple amplicons whose size distribution varies from strain to strain and which can be separated by gel electrophoresis
- eg 'PCR-ribotyping'
  - Can be used in *Staph. aureus* – initial investigation to show that isolates are not closely related or need further investigation (PFGE)

Relatively quick but quite difficult to standardise between laboratories

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Pulsed Field Gel Electrophoresis

Extract DNA and cut with specific restriction enzyme to give characteristic pattern of fragment sizes

Choice of enzymes – large or small fragments

- Small fragments (REFP) – easier to separate but less standardised
- Large fragments – need special equipment to separate (PFGE)

Much work done in standardising preparation and separation conditions

Still fairly slow (2-3 days) but *de facto* standard for many organisms now

- eg *E .coli* O157, *Staphylococcus aureus*

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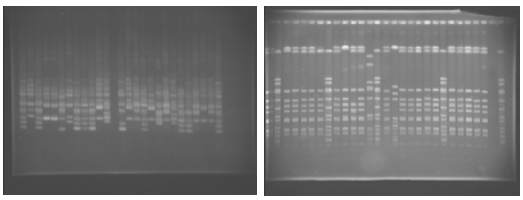
Gel Electrophoresis Images



PCR-ribotyping | Pulsed Field Gel Electrophoresis

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Problems with Gel Interpretation

Comparison between labs (and between gels within labs)

What differences are significant

- 'Tenover criteria' for PFGE
- Local analysis of patterns and epidemiology

Describing and designating patterns

Standardisation and computer analysis overcoming some difficulties

- National and International cooperation (PulseNet)

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Sequence-Based Typing

Automatable process (computer analysis necessary)

'Digital' results - easier comparison between labs

More expensive (at present)

Can choose level of discrimination

- Coarse – multiple stable genes – look at long term evolutionary trends
- Finer – fewer, variable gene(s) - outbreak investigation / local surveillance

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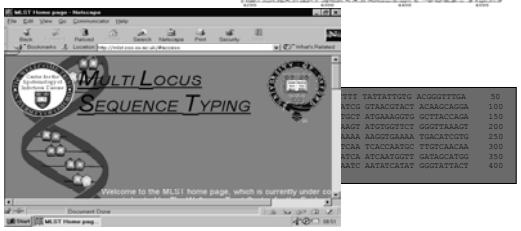
Multi-Locus Sequence Typing

7 Housekeeping genes



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Multi-Locus Sequence Typing (2)

Systems developed for many clinically important bacteria

- *Staphylococcus aureus*
  - Many isolates are typed so MLST not used for all
  - Not very useful in outbreaks (eg all EMRSA15's same)
- *Neisseria meningitidis*
  - Few isolates - possible to type high proportion
  - Can occasionally type without successful culture
  - Investigation of vaccine effects
- *Streptococccus pneumoniae*
- and others

Centrally maintained database (requires central funding)

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Single Gene Sequence Typing

Cheaper because less sequencing

Many of advantages of MLST (portable, automatable)

Usually used for finer discrimination than MLST

More variable gene chosen

- May correspond to serotyping antigen
  - *porA* in meningococci
- *spa* gene in *Staph. aureus*
  - new data promising

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A Comment on Common Types

Easier to recognise outbreaks of fairly unusual strains

Many bacteria will have some types that are so common that no typing method is usefully discriminatory.

eg EMRSA15, PFGE type PF15a in Scotland

Salmonella Enteritidis Phage Type 4 in Britain in most of 1990's

May reflect a real problem in the early spread of a successful lineage - there is not enough diversity to use for typing

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Final Comment on Interpretation

No available typing method is so good that its results can be taken out of their epidemiological context.

If the 'on the ground' epidemiology doesn't agree with the typing methods don't assume that either is automatically right – think again (and again and again).

You need to understand both the typing and the epidemiology – if you don't know enough – then find someone who knows more.

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