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Methicillin-resistant *Staphylococcus aureus* from China characterized by digestion of total DNA with restriction enzymes

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SUMMARY

A series of clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) from two hospitals in China was examined. Fragment patterns obtained by digestion of total cellular DNA with restriction enzymes were used to characterize the isolates, in combination with phage-typing, antibiotic resistance profile, and plasmid profile. Digestion of total cellular DNA with restriction enzymes was most useful in discriminating between isolates and yielded additional information on the relatedness of non-identical isolates. In one hospital a single strain, resistant to a large number of antibiotics, had apparently become endemic. In the second hospital a number of distinct but related strains were present. The isolates were also related but not identical to the strain of MRSA endemic at the London Hospital.

INTRODUCTION

Methicillin resistant strains of *Staphylococcus aureus* (MRSA) continue to be a problem for hospitals throughout the world. MRSA infections are generally hospital-acquired, frequently affecting patients who have undergone surgery or invasive procedures, or who have skin damage caused, for example, by burns. It is important in the management of an infected patient to understand the propensity of the infecting organism to cause cross-infection of other patients, and the potential consequences of such an event. Beyond the classification possible in the routine laboratory determination of the epidemiology of an organism may require further characterization to follow the spread of particular strains within a species. Isolates of *S. aureus* have long been typed by their susceptibility to lysis by a series of bacteriophages, and this has been invaluable in documenting the spread of particular strains (1). However MRSA may be non-typable by the international set of phages. In such situations a method of typing otherwise similar strains is important in identifying isolates requiring particular care to prevent their establishment in previously unaffected environments. Several new techniques based on electrophoresis have recently been used to type bacterial

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species, and most of these have been applied to MRSA (2, 3). We have found that restriction enzyme digestion of chromosomal DNA is a good discriminatory method (3). The incidence of MRSA in hospitals in Shanghai, China has increased greatly in the last few years, and is now causing severe treatment problems. To establish whether one particular strain of MRSA is involved, or whether multiple strains are causing infection, a collection of isolates was characterized by phage type, antibiotic resistance profile, plasmid profile, and restriction pattern of total cellular DNA.

METHODS

Bacterial isolates

Clinical MRSA isolates were received from China as agar stabs. Series A were from Hua Shan Hospital, collected May 1987–Dec 1987. Series B were from the Burns Unit, Rei jin Hospital, Shanghai, collected Dec 1986–Oct 1987. On arrival the isolates were subcultured onto blood agar then stored at −70 °C in peptone water containing glycerol and glucose 1% w/v; they were subcultured by overnight incubation on blood agar immediately before use. The 'Oxford' staphylococcus, NCTC 6571, and a clinical isolate of MRSA (EMRSA-1) from the London Hospital were included for comparison.

Characterization of isolates

Isolates were sent to the Staphylococcus Reference Laboratory at Colindale for phage typing. The antibiotic susceptibility of all isolates was tested by disk diffusion against penicillin (P), erythromycin (E), tetracycline (T), fucidic acid, gentamicin (G), methicillin (M), chloramphenicol (C), neomycin (N), rifampicin (R), vancomycin, and clindamycin (D). Plasmid DNA extractions, total DNA extractions, restriction digestion and electrophoretic analysis of DNA were performed as previously described (3). Briefly, plasmids were extracted by the method of Bennett *et al.* (4), using lysostaphin and lysis in alkaline SDS with extraction of the cleared lysate with phenol. Separation was in 0·8% agarose gels in TBE. Total DNA was prepared by multiple phenol and phenol/chloroform extraction of lysostaphin treated cells, followed by spooling of high molecular weight DNA from an ethanol precipitate. Electrophoresis was performed in 0·9% agarose gels in TBE. Dice coefficients (5) to measure the similarity between banding patterns were calculated as

$$\frac{\text{No. shared bands} \times 2 \times 100}{\text{Total no. bands in 2 samples}}$$

RESULTS

Initial characterization of isolates

Fifty clinical isolates of MRSA from two hospitals in China were phage typed by the Staphylococcus Reference Laboratory at Colindale. Ten representative isolates from each hospital were then chosen for further characterization; the phage typing patterns are given in Table 1. All of series A (Hua Shan) were non-typable by the International Set of phages at RTD × 100 and with the additional

Table 1. *Phage-type, antibiotic resistance profile and plasmid content of isolates*

Isolate	International phage set	Additional phages	Antibiotic resistance	Plasmid content
A1	NT*	NT	D E G P M C N R T	3·0, 4·6
A2	NT	NT	D E G P M C N R T	3·0
A3	NT	NT	D E G P M C N R T	3·0, 5·5
A4	NT	NT	D E G P M C N R T	3·0
A5	NT	NT	D E G P M C N R T	3·0
A6	NT	NT	D E G P M C N R T	3·0
A7	NT	NT	D E G P M C R T	4·6
A8	NT	NT	D E G P M C N R T	3·0
A9	NT	NT	D E G P M C N R T	3·0
A10	NT	NT	D E G P M C N R T	3·0
B1	Group III	83C	D E G P M T	2·2, 4·5
B2	NT	90, 932	G P M C R T	3·0, 4·5
B3	NT	932	D E G P M C R T	3·0
B4	NT	88A, 90, 932	G P M C T	3·0
B5	Group III	88A, 83C	D E G P M T	2·2, 4·5
B6	NT	932	D E G P M R T	4·5
B7	NT	90, 932	G P M C R T	3·0, 4·5
B8	NT	NT	D E G P M C R T	4·6
B9	NT	90, 932	D E G P M C T	2·2, 3·0, 3·6, 4·5
B10	NT	NT	D E G P M C R T	4·6

Phage types given at RTD × 100. Group III isolates B1 and B5 are distinct (see text). All isolates were susceptible to fucidic acid and vancomycin (see Methods for antibiotic abbreviations). Plasmid sizes given in kilobase pairs.
* NT, not typable.

phages, although A7 gave a weak reaction with phage 932. Only B1 and B5 of series B (Rei jin) were typable by the International Set at RTD × 100, but in some isolates inhibition reactions were recorded. The additional phages produced a range of typing patterns among series B isolates (Table 1); B3 and B6 and nine other B isolates were lysed by 932 only, B2, B7 and B9 by 932 and 90 and B4 by 88A, 932 and 90. B8 and B10 and eight other B isolates gave no reaction with any of the phages used.

Antibiotic susceptibility

Susceptibility of the 20 selected isolates to a range of antibiotics was tested by the disk diffusion method; results are shown in Table 1. Series A isolates were all susceptible to fucidic acid and vancomycin, but resistant to clindamycin, erythromycin, gentamicin, penicillin, methicillin, chloramphenicol, rifampicin, and tetracycline, and all except A7 resistant to neomycin. Series B were generally less resistant and had more variable profiles than series A (Table 1). B1 and B5 were resistant to methicillin, penicillin, tetracycline, erythromycin, clindamycin and gentamicin, with B3, B6, B8, B9 and B10 additionally resistant to chloramphenicol and/or rifampicin. B2, B4 and B7 had similar profiles but were not resistant to erythromycin or clindamycin. None of series B was resistant to fucidic acid, neomycin or vancomycin.

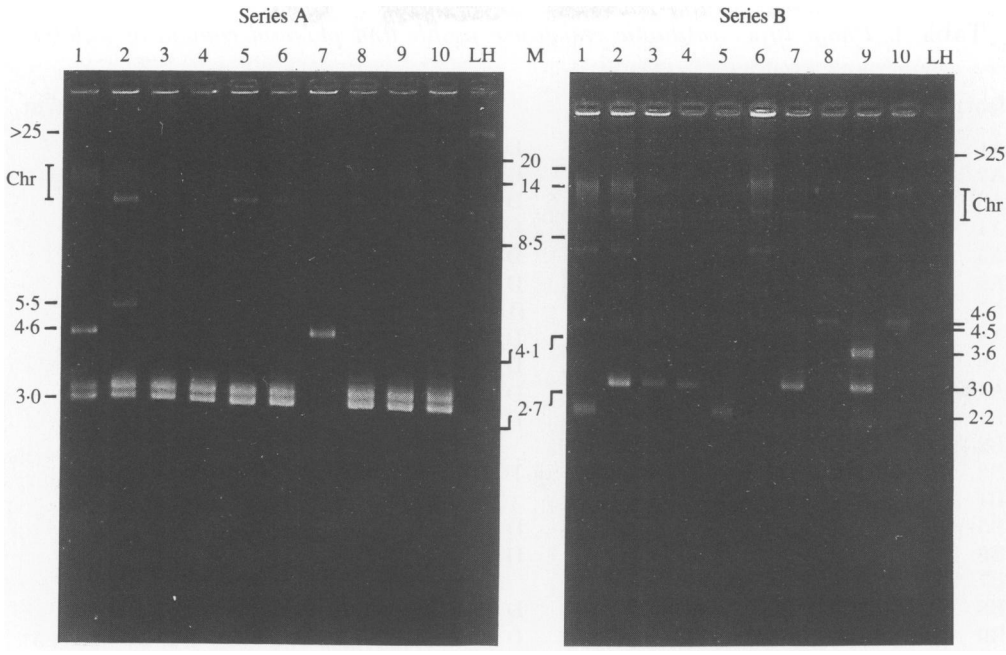

Fig. 1. Plasmid DNA isolated from Chinese MRSA isolates and a London Hospital EMRSA carrying a large gentamicin resistance plasmid (LH). The positions of closed circular marker plasmids of known size run in parallel are indicated in lane M. Calculated sizes (in kilobase pairs) of closed circular forms detected are given.

Plasmid profiles

Plasmid extractions performed on the isolates (Fig. 1) were analysed (Table 1). Again the A isolates presented a relatively homogeneous series. All except A7 carried a plasmid of 3 kb, A1 and A7 had plasmids of 4·6 kb, and A2 had a further plasmid of 5·5 kb. In series B plasmids of 3 kb were detected in B2, B3, B7, and B9, and plasmids of 4·6 kb in B8 and B10. A small plasmid of 2·2 kb, which could correspond to a cryptic plasmid observed in some MRSA strains (6), was found in B1, B5 and B9. A further plasmid of 4·5 kb was carried by B1, B2, B5, B6, B7 and B9, and B9 had a fourth plasmid of 3·6 kb (Table 1). No large plasmids of greater than 20 kb were detected in the Chinese isolates (Fig. 1).

Restriction enzyme digestion of total cellular DNA

Preparations of total cellular DNA were digested with the restriction enzymes *Bgl*II or *Sal*I, and the fragments produced analysed by gel electrophoresis. Banding patterns obtained by digestion with *Bgl*II and *Sal*I are illustrated in Figures 2 and 3 respectively. On digestion with *Bgl*II (Fig. 2) series A isolates formed two groups; eight of the isolates had patterns indistinguishable from each other, while A1 and A7 differed by the presence of a number of extra bands, of which most were identical between these two isolates. After digestion with *Sal*I (Fig. 3), A1, A7 and also A5 differed in a small number of bands from the common pattern held by the bulk of the A series.

Analysis of the B series digestion patterns showed that with both enzymes the majority of bands were held in common between all isolates (and many also in

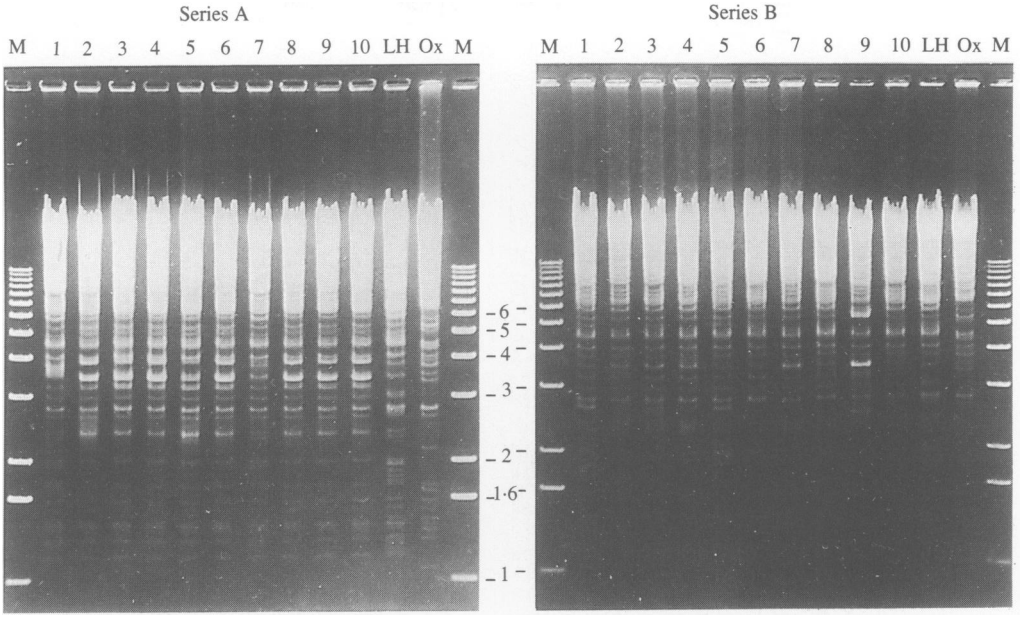

Fig. 2. Total cellular DNA preparations from Chinese MRSA isolates, London Hospital EMRSA-1 (LH) and the Oxford staphylococcus (Ox) digested with restriction endonuclease *Bgl*II. Molecular size markers were included (M), and sizes given in kilobase pairs.

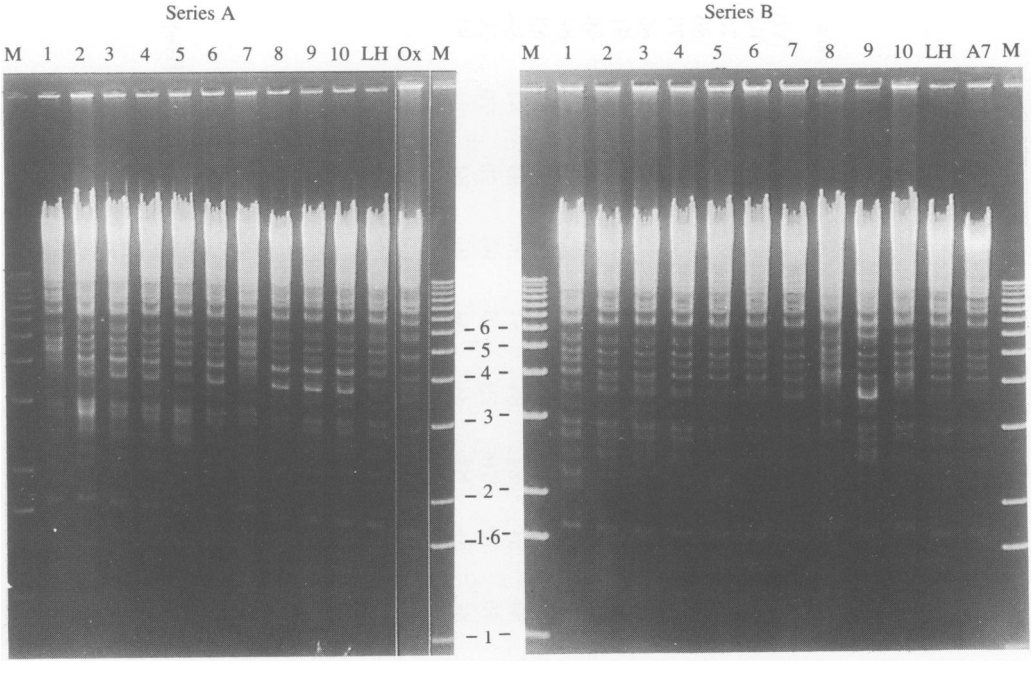

Fig. 3. Total cellular DNA preparations from Chinese MRSA isolates, London Hospital EMRSA-1 (LH) and the Oxford staphylococcus (Ox) digested with restriction endonuclease *Sal*I. Molecular size markers were included (M), and sizes given in kilobase pairs.

Table 2. *Dice coefficient of similarity in* Bg*lII fragment patterns between isolates*

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	LH
Ox	58	65	65	65	65	65	63	65	65	65	58	58	58	61	58	59	59	59	58	59	55
LH	86	75	75	75	75	75	86	75	75	75	91	86	86	81	91	88	88	88	86	88	
B10	98	86	86	86	86	86	98	86	86	86	98	93	98	92	98	100	95	100	93		
B9	90	79	79	79	79	79	90	79	79	79	95	95	95	90	95	93	98	93			
B8	98	86	86	86	86	86	98	86	86	86	98	93	98	92	98	100	95				
B7	93	81	81	81	81	81	93	91	91	91	98	98	93	92	98	95					
B6	98	86	86	86	86	86	98	86	86	86	98	93	98	92	98						
B5	95	84	84	84	84	84	95	84	84	84	100	95	95	90							
B4	95	83	83	83	83	83	95	83	83	83	90	90	90								
B3	95	84	84	84	84	84	95	84	84	84	95	90									
B2	90	79	79	79	79	79	90	79	79	79	95										
B1	95	84	84	84	84	84	95	84	84	84											
A10	84	100	100	100	100	100	89	100	100												
A9	84	100	100	100	100	100	89	100													
A8	84	100	100	100	100	100	89														
A7	95	89	89	89	89	89															
A6	84	100	100	100	100																
A5	84	100	100	100																	
A4	84	100	100																		
A3	84	100																			
A2	84																				

Ox, Oxford staphylococcus; LH, London Hospital MRSA. Fragments of 1·4–6 kb were compared.

common with the A series patterns). Yet there were seven discernible patterns after digestion with *Bgl*II, and four patterns after digestion with *Sal*I.

Combining the results with the two enzymes, A2, A3, A4, A6, A8, A9, and A10; B1 and B5; and B6, B8 and B10 each formed groups within which isolates could not be distinguished, while the remaining eight isolates were each distinct by at least one band difference with at least one of the restriction enzymes used (Figs 2 and 3).

The A series and B series digestion patterns were compared with patterns obtained with the MRSA strain endemic in the London area (the EMRSA, ref 7, now EMRSA-1), and with the 'Oxford' staphylococcus. On digestion with *Sal*I 15 or 16 out of the 16 bands detectable in the London MRSA between 1·6–6 kb in size were held in common by all the Chinese MRSA, but most had between one and three additional bands. Isolates A5 and B4 were identical to the London EMRSA-1 on digestion with this enzyme. However, digestion with *Bgl*II yielded patterns in which the London strain was always distinguishable, with between three and ten bands differing in Chinese isolates. The 'Oxford' strain had many more band differences from all MRSA, although again more bands were held in common after digestion with *Sal*I than with *Bgl*II.

The degree of similarity between the banding patterns of the isolates examined was calculated as a Dice coefficient (5), relating the number of common bands to the total number of bands (Table 2). A complete table of comparison was calculated only for *Bgl*II digestion patterns, since this enzyme gave greater discrimination between isolates. To prevent problems of interpretation in dense or faint areas of the gel, only bands of 1·4–6 kb were included in the calculations. A2–A6 and A8–A10 all gave identical banding patterns with *Bgl*II and thus had 100% similarity. The patterns derived from the B isolates and A1 and A7 were also closely related to each other, having similarity values of 90% or more. All Chinese isolates also had a higher degree of similarity to the MRSA from the London Hospital (75–91%) than to the Oxford staphylococcus (58–65%) (Table 2). Calculations with the patterns produced by *Sal*I digestion yielded closer relationships, but generally ranked in the same order.

DISCUSSION

MRSA isolates from Hua Shan Hospital

The isolates from Hua Shan Hospital, A1–A10, formed a rather homogeneous group by all methods of comparison. The ten isolates were uniformly non-typable by the international set of typing phages, and only A7 gave a weak reaction with one of the four additional phages.

Antibiotic susceptibility testing revealed that all isolates were resistant to the majority of first and second line antibiotics, being susceptible only to vancomycin and fucidic acid of the 11 agents tested; A7 again differed in being susceptible to neomycin. Examination of plasmid profiles of the isolates demonstrated a plasmid of 3 kb to be present in all isolates except A7, while A1, A2 and A7 carried larger plasmids.

Digestion of chromosomal DNA with restriction enzymes has proved very useful in characterizing MRSA isolates in the UK (3). In the present study two

different restriction enzymes were used to generate banding patterns. The results confirmed that the majority of isolates from Hua Shan Hospital were indistinguishable. However isolates A1, A5 and especially A7 all had digestion patterns which were distinct from the majority and from each other, although clearly related. It is suggested that the majority pattern represents a strain endemic in the hospital and acquired by susceptible patients either from the environment, or from staff or other patients carrying the organism asymptomatically or symptomatically. Care should be taken to prevent the further spread of this strain, which is resistant to a particularly extensive list of antibiotics. The variants might also be endemic at lower frequencies, or have been introduced to the hospital for instance by transfer of patients or staff from other hospitals (see below).

MRSA from Rei jin Hospital (Burns Unit)

In contrast to the isolates from Hua Shan Hospital, the B series of isolates were rather heterogeneous in character. Isolate B1 had phage type (at RTD × 100) 6/42E/47/54/83A/85/81 plus 83C, and isolate B5 had phage type (at RTD × 100) 79/95/6/42E/47/53/54/75/83A/84/85/81 plus 88A/83C. These two isolates differ from each other by the criterion of two strong differences (see ref. 1), and from the remainder of the B series which were non-typable with the international set of phages (Table 1), although some showed inhibition reactions at RTD × 100. All of the isolates except B8 and B10 were lysed by at least one of the additional phages, with six types being discernible (Table 1).

The B isolates were resistant to multiple antibiotics, but the profiles varied and none were resistant to as many agents as the majority of the A series. Six different combinations of resistance were observed. Plasmid profiles were also more complex than for the A isolates with five different plasmids detected in total.

The pattern of fragments produced by digestion of total cellular DNA with either *Bgl*II or *Sal*I showed that all isolates have most bands in common, and must be related, but small differences were observed. Interestingly the patterns were more similar to those of the odd members of the A series than to the pattern common to most A isolates. The epidemiology of MRSA at Rei jin Hospital is clearly complex, with infection being caused by several different strains. Most patients at this hospital, suffering from burns, would be very susceptible to infection by *S. aureus*; it is possible that this reduces the selection pressure on the organism and allows multiple strains to occupy that ecological niche which favours resistant organisms.

Restriction enzyme digestion of total cellular DNA as a method for characterizing MRSA

In the present study digestion of total cellular DNA with restriction enzymes has proved useful in characterizing a series of MRSA from Shanghai. The overall picture that the majority of isolates from Hua Shan Hospital are identical while isolates from Rei jin Hospital are more diverse is confirmed by all methods of characterization applied, although differences in detail occur. Restriction enzyme digestion of total DNA was particularly useful for the phage non-typable isolates

since this group could be further subdivided, and a positive result was obtained which will be available for subsequent comparison to other isolates.

Perhaps the most interesting aspect of the method is that it permits the relative relatedness of isolates to be compared (Table 2). Thus on the basis of the number of bands held in common in *Bgl*II digests the B isolates (from Rei jin) and the atypical A isolates (from Hua Shan) are more closely related to each other than to the typical Hua Shan A isolate. Furthermore all the Chinese isolates have many bands in common with the MRSA strain endemic at the London Hospital, but the typical A isolate, apparently endemic at Hua Shan, is actually less like the London strain than are the B isolates. Indeed the B and atypical A isolates are as similar to the London MRSA as to the typical A isolate. In contrast, all of the MRSA isolates have a much lower similarity to the Oxford staphylococcus.

It must be emphasized that it is the similarity of the banding pattern that is being compared, reflecting conservation of restriction sites in the genome. The percentage similarity of bands is related to, but not directly proportional to, overall sequence similarity. In particular the two enzymes chosen for this study have less sites in *S. aureus* DNA than would be expected in a random sequence, so factors such as the restriction-modification system of the organism may be affecting the evolution of the banding pattern. Furthermore plasmid DNA may also contribute to the banding pattern. Careful comparison of plasmid content with the total DNA digestion pattern indicates that in this study no extra bands are attributable to plasmid DNA, but this possibility needs to be considered. At the present time the significance of relationships between strains is difficult to assess, but characterization of a wide variety of strains by digestion of total DNA could produce a valuable database with which to compare new isolates and strains of known origin.

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REFERENCES

1. Parker MT. The significance of phage-typing patterns in *Staphylococcus aureus*. In: Easmon CSF, Adlam C, eds. Staphylococci and staphylococcal infections. Academic Press, 1983: 33–62.
2. Gaston MA, Duff PS, Naidoo J, Ellis K, Roberts JIS, Richardson JF, Marples RR, Cooke EM. Evaluation of electrophoretic methods for typing methicillin-resistant *Staphylococcus aureus*. J Med Micro 1988; **26**, 189–97.
3. Jordens JZ, Hall LMC. Characterisation of methicillin-resistant *Staphylococcus aureus*. J Med Micro 1988; **27**: 117–23.
4. Bennett PM, Heritage J, Hawkey PM. An ultra-rapid method for the study of antibiotic plasmids. J Antimicrobial Chemotherapy 1986; **18**: 421–4.
5. Dice LR. Measures of the amount of ecological association between species. Ecology 1945; **26**: 297–302.
6. Townsend DE, Ashdown N, Bolton S, Bradley J, Duckworth G, Moorhouse EC, Grubb WB. The international spread of methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 1987; **9**: 60–71.

7. Marples RR, Richardson JF, de Saxe MJ. Bacteriological characters of strains of *Staphylococcus aureus* submitted to a reference laboratory related to methicillin resistance. J Hyg 1986; **96**: 217–23.