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PHAGE TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Summary

448 isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimens of patients from the University Hospital, Kuala Lumpur, were phage-typed. These included 35 strains causing two separate outbreaks of infection, one in surgical Ward 6B and another in the Special Care Nursery (SCN). Antibiograrns of these outbreak strains in Ward 6B and SCN were entirely different. Phage-typing revealed that 72% of the MRSA isolates were typable. They were typed entirely by Group III phages, the majority (76%) of which were phage type 85. There was only one isolate in SCN which was typed by Group I (phage 80) and Group III phages. None were typed by phages 94, 95, 96 and Group II phages. 14.6% of the typable isolates gave the long pattern reaction of the phage 6/47/54/75/77/83A/84/85 complex. The majority of the outbreak strains in Ward 6B were of phage type 85, whereas those in the SCN were all of the 6/47/54/75/77/83A/84 phage pattern with the exception of one isolate which was also typed by phage 80, a Group I phage.

Keywords: Nosocomial infections, infection outbreak.

INTRODUCTION

Methicillin resistance in strains of *Staphylococcus aureus* was detected shortly after the introduction of the first penicillinase-resistant penicillin, -methicillin-in 1961.[1] There was increasing concern about the international spread of MRSA by 1980.[2,3] Phage-typing is a method of classification based on the susceptibility of *Staphylococcus aureus* to a set of phages chosen to make as many epidemiologically valid distinctions as possible between strains. It can be applied when studying the origin and spread ofstaphylococcal infections in an outbreak.

This paper describes the phage typing of 448 isolates of MRSA from clinical specimeris of patients in the University Hospital. Kuala Lumpur, from January 1988 till December 1989.

MATERIALS AND METHODS

All 448 isolates in this study were obtained from in-patients of the University Hospital. Kuala Lumpur between January 1988 and December 1989. They had been identified as MRSA by coagulase and methicillin disc sensitivity testing (10ug per disc, Mast Laboratories Ltd., United Kingdom). Strains of MRSA associated with outbreaks of nosocomial infections in patients were also phage-typed for comparison. 21 isolates were from different patients of surgical Ward 6B and 14 isolates were from the paediatric Special Care Nursery (SCN). The isolates from Ward 6B were resistant to erythromycin, tetracycline, co-trimoxazole and gentamicin while those from the SCN were all sensitive to erythromycin, tetracycline and co-trimoxazole. 23 propagating strains of *Staphylococcus aureus* of known lytic spectrum with the international set of phages were used as control organisms.

Media

Staph typing agar consisting of Oxoid nutrient broth no. 2, sodium chloride, Oxoid agar no. 1 with the addition of calcium chloride was used. They were dried for 60 minutes at 37°C before use.

Phages and phage-typing

The international basic sec of 23 typing phages was used and they arc listed as follows:

Group I	:	29	52	52A	79	80
Group II	:	3A	3C	55	71	
Group III	:	6	42E	47	53	54
		75	77	83A	84	85
Miscellaneous	:	81	94	95	96	

(This set has remained unchanged since 1974)

The phage-typing was performed by tlie standard method of Parker.[4] The phages liad been propagated by the soft-agar layer method

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of Swanstrom and Adams.[5] Test strains of ***Staphylococcus aureus*** were suspended in nutrient broth, incubated for 4 hours at 37°C and lawned on the staph typing agar. The plates were allowed to dry at room temperature. Phages were then applied onto the media using a Lidwell typing machine, in a standard arrangement at the routine test dilution (RTD) which is the highest dilution of a phage which just fails to give confluent lysis on the homologous propagating strains. Higher phage titres of 100RTD and 1000RTD were also used when the test strains could not be typed at RTD. The phage drops were allowed to dry and the plates incubated at 30°C for 18 hours.

Phage reactions (lysis) were read with the aid of a X10 hand lens and recorded as follows:

± = 1 – 19 plaques

\+ = 20 – 50 plaques

++ = >50 plaques

CL = confluent lysis

O = inhibition reaction

Only reactions producing confluent lysis and weak lytic reactions (++ and +)were taken into account. Any ± reactions were taken as non-typable results. Inhibition reactions, which occurred as thinning of the lawn in the areas of the phage spot, were not considered as positive results. A test strain may be lysed by more than one phage, and the strains were characterised by the patterns of lysis by the phages used.

RESULT

Of a total of 448 isolates of MRSA, 321 (72%) were typable. 127 (28%) remained non-typable even at phage titres of 1000RTD. Of the 321 typable isolates, only 95 (30%) could be typed at the RTD although typability increased to 70% (226 isolates) when the phage titres were increased to 100RTD.

All the isolates were lysed entirely by Group III phages (Table 1). None were typed by phages 94, 95, 96 and Group II phages. At least 4 phage patterns were seen in this study. The majority (244 or 76% of isolates) were typed by phage 85 only (Table 2). 9% of typable isolates were typed with phages 83A/85. The remaining 47 typable isolates gave the long pattern reactions, of which a third (14) were the 6/47/54/75/77/83A/84/85 phage type. 16 (76.2%) of 21 isolates of MRSA from Ward 6B patients were typed by phage 85 only. The rest were non-typable. Interestingly, MRSA isolates from the SCN gave the long pattern reaction with Group III phages, that is, the 6/47/54/75/77/83A/84 phage type. There was only one isolate from the SCN which was also typed by phage 80 (Group I phage) in addition to lysis by the above Group III phages.

DISCUSSION

The phage typing technique is useful for acquiring knowledge of the frequency of various phage types of staphylococci in the population. Additional experimental phages, namely 88A, 90, 83C and 932 are available for optional use and have been found to increase typability of many MRSA which are

TABLE 1
PHAGE-TYPING OF 321 MRSA ISOLATES

Phage	Number of isolates lysed (%)
80	1 (0.3)
85	306 (95.3)
83A	56 (17.4)
84	33 (10.3)
47	22 (6.8)
54	21 (6.5)
77	15 (4.7)
75	7 (2.2)
6	6 (1.9)
81	5 (1.6)
42E	2 (0.6)

TABLE 2
PHAGE-TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*, UNIVERSITY HOSPITAL, KUALA LUMPUR

Phage type	No. of isolates (%)
85 only	244 (76.0)
83A/85	30 (9.4)
6/47/54/75/77/83A/84/85	47 (14.6)
Total	321 (100.0)

non-typable by the standard international set of 23 phages.[6,7] Phage typing of the MRSA isolates in this study showed that they were entirely typed by Group III phages. In the United Kingdom, strains of MRSA which are highly transmissible have been termed epidemic MRSA (EMRSA) to differentiate them from other MRSA (OMRSA) strains.[6] The EMRSA were often typed by phage Group III, especially phage 85, occasionally by phages 84 and 83A, and also by experimental phages 88A and 932 but many were found to be untypable. Other authors have also reported that resistant strains of ***Staphylococcus aureus*** often fell into Groups I + III or Group III in typing patterns.[1,7] Lytic Groups II and V (phages 94/96) strains were poorly represented by MRSA isolates.

Phage-typing of the MRSA associated with outbreaks of nosocomial infections by this organism in surgical Ward 6B and the SCN revealed that they were distinguishable strains. Those in Ward 6B were typically phage type 85 while the isolates in the SCN gave the long pattern reactions by the Group III phages 6/47/54/75/77/83A/84. When their antibiotic susceptibility patterns were compared, all the isolates in Ward 6B were characteristically resistant to .erythromycin, tetracycline, co-trimoxazole and gentamicin, whereas those of the SCN were sensitive to erythromycin, tetracyline and co-trimoxazole.

In conclusion, phage typing is a useful technique and is successful as one of the methods of strain characterisation of ***Staphylococcus aureus,*** other than antibiotic susceptibility patterns by minimum inhibitory concentrations (MICs), coagulase typing and plasmid typing. Evaluation of MICs and plasmid analysis, together with phage typing, are useful epidemiological tools but they are all too time-consuming and tedious to be done routinely in the microbiology laboratory. The disadvantage of phage typing is that the set of phages in use at present gives only pattern reactions, rather than truly type-specific reactions and one would need specialised training in this technique for the propagation and maintenance of phages at high titres. Furthermore, phage typing has to be carefully standardized, as it appears to be affected by variations in media, inoculum size and environmental factors. However, the technique of phage typing is simple to perform and is useful particularly for epidemiological studies of outbreaks of infections by ***Staphylococcus aureus.*** Since MRSA is a problem in many hospitals, being associated with outbreaks of infections, creating difficulties in antibiotic therapy due to its multiple resistance, it is of utmost importance to prevent its spread by careful infection control procedures. Typing of MRSA is not necessary for management of the patient but provides useful information for characterisation of outbreak strains and for determining the evolution of the properties of ***Staphylococcus aureus.***

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REFERENCES

1. Jevons MP. Celbenin-resistant staphylococci. Br Med J 1961; 1: 124–5.
2. Shanson DC. Antibiotic-resistant ***Staphylococcus aureus.*** J Hosp Infect 1981; 2: 11–36.
3. 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.
4. Parker MT. Phage-typing of ***Staphylococcus aureus*** In: Norris JR, Ribbons DW, eds. Methods in Microbiology. London Academic Press, 1972; 78: 1–28.
5. Swanstrom M, Adams MH. Agar layer method for production of high titre phage stocks. Proc Soc Exp Biol (NY) 1951; 78: 372–5.
6. Cookson BD, Phillips I. Epidemic methicillin-resistant ***Staphylococcus aureus.*** J Antimicrob Chemother 1988. 21 (Suppl C): 57–65.
7. Marples RR, Richardson JF, deSaxe MJ. Bacteriological characters of strains of ***Staphylococcus aureus*** submitted to a reference laboratory related to methicillin resistance. J Hyg (Camb) 1986; 96: 217–23.
8. Parker MT, . Asheshov EH, Hewitt JH, Nakhla LS, Brock BM. Endemic staphylococcal infections in hospitals. Ann New York Acad Science 1974; 236: 466–84.