METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) - PHAGE-TYPING OF MALAYSIAN AND INTERNATIONAL ISOLATES

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ABSTRACT

Twenty-one isolates of methicillin-resistant Staphylococcus aureus (MRSA) from Malaysia (M-MRSA) derived from various sources associated with nosocomial infections were phage-typed and compared with 54 international isolates associated with epidemic and sporadic episodes of infections. It appeared that the majority of M-MRSA were non-typable by the international basic set of phages. Two (9.5%) were typed by phage 85. Phage-typing of MRSA revealed that the strains were almost completely restricted to phage groups III and a lesser portion to phage groups I and III.

Keywords: Methicillin-resistant Staphylococcus aureus, phage-typing

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INTRODUCTION

Staphylococcus aureus is an important hospital pathogen and has been known to cause serious hospital-acquired infections, especially in the surgical, orthopaedic and burn units. The emergence of multiple-antibiotic-resistant *Staphylococcus aureus* strains, which are resistant to methicillin, has created new problems and international spread of these strains have occurred in recent decades. Epidemic MRSA infections are common in hospitals, mostly large referral hospitals, in France, Germany, Austria, Italy, the Middle East, Greece, the United States of America, United Kingdom and Australia[1,2].

Phage-typing has been successful as a method of strain characterisation in *Staphylococcus aureus* and it can be applied when studying the spread of staphylococcal infection and its origin in an outbreak[3]. "Reverse-typing" has also been used for strain discrimination after induction of phages from lysogenic strains of *Staphylococcus aureus* by mitomycin C[4].

This paper describes the phage-types of 21 isolates of MRSA from Malaysia, and 54 international isolates.

MATERIALS AND METHODS

Sources of MRSA isolates

All 75 isolates had been identified as MRSA by coagulase testing, biochemical tests and methicillin disc sensitivity tests. They were grouped into 4 groups A, B, C and D.

Group A (M-MRSA) comprised of 21 isolates from Malaysia at the University Hospital, Kuala Lumpur. Ten isolates were from infected patients, one was from a nasal carrier and 10 isolates were from the hospital environment. The other 54 isolates were obtained from the Central Public Health Laboratory Service, Colindale, London, England, and they were grouped under groups B, C and D. Group B (IE-MRSA) consisted of 20 epidemic strains referred to as the International Epidemic MRSA. Group C (IS-MRSA) consisted of 21 isolates causing sporadic infections and they were referred to as the International Sporadic MRSA.

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The 41 international MRSA isolates in Group B and Group C were obtained from the following countries : Denmark 2, Canada 2, Yugoslavia 2, Austria 1, United States of America 18, Saudi Arabia 1, Iraq 1, Norway 2, Italy 4, Hungary 1, China 2, Hong Kong 4 and Singapore 1.

Group D (UK-MRSA) had 13 isolates of epidemic MRSA in the United Kingdom. A standard MRSA ST84/5518, an isolate of the Thames Epidemic MRSA was used as a control organism.

Media

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

Phage and phage-typing

The international basic set of 23 typing phages were used.

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)

In addition, 4 experimental phages were included: 88A, 90, 83C and 932. The phage-typing was performed by the standard method of Parker[5]. The phages had been propagated by the soft-agar layer method of Swanstrom and Adams[6]. The phages were applied using a Lidwell typing machine, in a standard arrangement at the routine test dilution (RTD), 100RTD and 1000RTD on to plates of Staph typing agar lawned with the test strain of *Staphylococcus aureus* which had been suspended in nutrient broth and incubated for 4 hours at 37°C. The routine test dilution is defined as the highest dilution of a phage which just fails to give confluent lysis on the homologous propagating strains.

Experimental phages were used at 100RTD and 1000RTD only. The phage drops were allowed to dry and the plates were incubated at 30°C for 18 hours. Phage reactions (lysis) were read with the aid of X10 hand lens and were recorded as follows:

+	=	1-19 plagues
+	=	20-50 plagues
++	=	> 50 plagues
CL	=	confluent lysis
O	=	inhibition lysis

Only reactions producing confluent lysis (CL) and weak lytic reactions (++ and +) were taken into account. Any ± reactions were taken as non-typable results. Inhibition reactions, which occur as thinning of the lawn in the areas of the phage spot, was not considered as positive reactions.

RESULTS

Forty five (60%) isolates could be typed in this study. Table I shows the number of MRSA isolates in each group which were typable by one or more of the 27 phages used. Of the 75 isolates which formed the four MRSA groups studied, lytic groups II and V (phages 94/96) strains were not represented at all (Table II) .The percentages of typable strains lysed by each phage are shown in Fig. 1.

Table I: Phage-typing of 75 methicillin-resistant *Staphylococcus aureus* isolates

Phage typing	Number of isolates (%)				
	Group A M-MRSA n=21	Group B IE-MRSA n=20	Group C IS-MRSA n=21	Group D UK-MRSA n=13	Total n = 75
Non-typable	19(90.5)	5(25.0)	5(23.8)	1(7.7)	30(40.0)
Typable	2(9.5)	15(75.0)	16(76.2)	12(93.3)	45 (60.0)

Table II: Phage-types of 45 Methicillin-resistant *Staphylococcus aureus* isolates

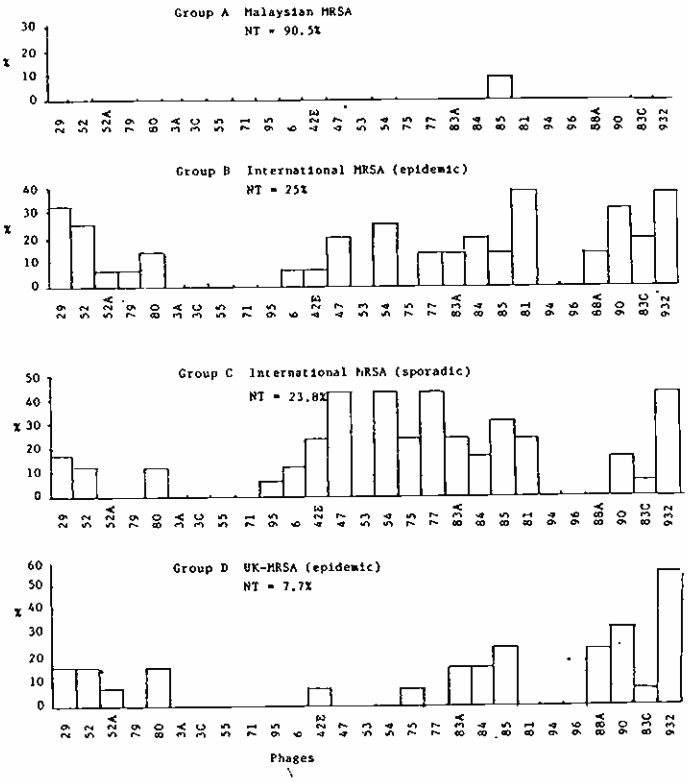
Lytic Group	Number of isolates (%)				
	Group A M-MRSA n=21	Group B IE-MRSA n=15	Group C IS-MRSA n=16	Group D UK-MRSA n=12	Total n=45
Group I	0	6(40.0)	4(25.0)	3(25.0)	13(28.9)
Group I + III	0	2(13.3)	3(18.8)	2(16.7)	7(15.5)
Group III	2(100.0)	7(46.7)	14(87.5)	8(66.6)	28(62.2)
Miscellaneous phage 81	0	6(40.0)	4(25.0)	0	10(22.2)
Experimental phage	0	9(60.0)	9(56.3)	7(58.3)	24(53.3)

All the 21 M-MRSA isolates were non-typable with the international set of phages at the routine test dilution (RTD). However, 2 (9.5%) isolates were typed strongly by phage 85, one at 100RTD and the other at 1000RTD. These isolates were from the Special Care Nursery from an infant with MRSA septic arthritis and septicaemia, and the other from the environment in a surgical ward. No lysis was seen at all with the experimental phages 88A, 90, 83C and 932.

Amongst the 20 international epidemic MRSA isolates (IE-MRSA) in group B, 15 (75%) isolates were typable. Eleven (55%) were untypable at RTD and 5 (25%) remained non-typable at 100RTD even to the experimental phages. Six (40%) were typed by phage Group I especially phage 29 in 5 (33%) isolates and phage 52 in 4 (26.7%) isolates. Nine (60%) isolates were typed by experimental phages, particularly phages 90 (33.3% of isolates) and 932 (40% of isolates). Three (20%) of the isolates were non-typable by the basic set of phages but could be typed by experimental phages only. There was one (6.6%) isolate which was typed by group I phage 29 only, 2 (13.3%) isolates typed by group I and phage 81, and 1 (6.6%) isolate by group I and experimental phages.

The MRSA isolates in group C (IS-MRSA) were mainly represented by phage group III, that is, in 14 (87.5%) of 16 typable strains, especially by phages 47 (43.7%), 54 (43.7%), 77 (43.7%), 85 (31.2%), experimental phages 932 (43.7%) and phage 90 (18.7%).

Fig 1 - Percentage of typable strains lysed by each phage NT, not typable



Of the 13 Group D (UK-MRSA) isolates, only one was untypable. Here again, the majority (66.6%) of the typable isolates were lysed by group III phages. Seven (58.3%) typable isolates reacted with the experimental phages, particularly phages 932 (58.3%), 90 (33.3%) and phage 88A(25%). Three (25%) isolates were typed by experimental phages only.

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* has been an important pathogen associated with nosocomial infections in many countries. In 1988, 13% of all *Staphylococcus aureus* isolated from patients at the University Hospital were found to be methicillin-resistant, although they were not entirely associated with clinically significant disease. Ten percent of all nosocomial infections was associated with MRSA, especially causing skin and wound infections, particularly in the surgical, burns and orthopaedic patients. These MRSA strains were multiply-antibiotic resistant to five or more antibiotics, namely penicillin, tetracycline, erythromycin, chloramphenicol, co-trimoxazole, gentamicin and other aminoglycosides. Treatment becomes problematic and drugs presently available against MRSA are limited. They are usually sensitive to vancomycin, which is costly and also potentially toxic.

Strain distinguishability of MRSA isolates is important in the epidemiological study of nosocomial infections[1,2,7].

Other typing profiles, namely phage-typing and plasmid typing are more reliable in strain differentiation, although these are technically difficult and costly to be done routinely in a hospital. In this study, phage-typing of MRSA isolates from Malaysia was rather unrewarding by the standard set of phages. Only 2 (9.5%) were typable by phage 85. Methicillin resistance in *Staphylococcus aureus* is almost completely restricted to strains of phage groups III and I + III. Fifty-three percent of the MRSA were typed with experimental phages, especially phage 932. Typing of the international MRSA by group III

phages were frequently recorded with phages 47, 54, 77 and 85. Lysis by phage 81 (miscellaneous group) was also frequently seen in the international MRSA strains, but this was not observed in the strains from United Kingdom. In the United Kingdom, highly transmissible strains of MRSA have been termed epidemic MRSA (EMRSA) to differentiate them from other MRSA (OMRSA) strains[7]. The EMRSA is often typed by phage group III especially phage 85, occasionally by phage 84 and 83A, and also the experimental phages 88A and 932, but many strains remain untypable.

The epidemic and sporadic strains could not be differentiated by phage-typing alone. For the phage group I strains, lysis by phage 29 was the commonest, followed by phages 52 and 80. In the groups B, C and D studied, 40-50% of the isolates were typed by experimental phages, which may lyse non-typable strains. These experimental phages were useful in typing outbreak strains which were not lysed by the basic-set phages.

The 75 MRSA isolates studied did not belong to phage groups II and V (94/96 phages) as such phage types are rarely methicillin-resistant. Non-typability had been reduced by 20-23% by typing at 100RTD, although this is not normally done when an adequate result is obtained at RTD.

Phage typing is useful in identifying the existence of a particular epidemic strain which have spread and caused outbreak. A larger number of MRSA isolates need to be studied to determine the significance of certain phage-types in relation to community or hospital-acquired infections.

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