EPIDEMIOLOGICAL TYPING

Lectin typing of methicillin-resistant *Staphylococcus aureus*

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This study reports the patterns of agglutination of 77 clinical isolates of methicillin-resistant *Staphylococcus aureus* by 32 commercially available lectins. Cell suspensions were not pre-treated. Each isolate was cultured on three media: Columbia blood agar, trypticase-soy agar and Chapman Stone agar. The lectins agglutinating each isolate varied widely depending on culture medium; only five isolates were agglutinated by the same set of lectins regardless of the culture medium used. Lectin typing could be a useful epidemiological tool, but it is necessary to standardise assay conditions (notably culture medium) to enable meaningful comparison of the results produced by different research groups or centres.

Introduction

Staphylococcus aureus, especially methicillin-resistant *S. aureus* (MRSA), is one of the most important causes of nosocomial infection. Furthermore, problems due to MRSA appear to be increasing, particularly in southern Europe, where the incidence of infection and rates of antibiotic resistance are alarmingly high. In Europe, the incidence of methicillin resistance among isolates of *S. aureus* from hospital patients ranges from <1% in Scandinavia to >30% in France and Italy [1]. In Spain, the incidence of methicillin resistance among hospital isolates of *S. aureus* has increased dramatically over recent years, from 1.5% in 1986 to 17.9% in 1996 [2], and to as high as 44% in some hospitals, giving rise to a serious epidemic situation [3]. The incidence of methicillin resistance among hospital isolates of *S. aureus* also appears to be on the rise in Japan [4].

The selectivity of lectins for microbial surfaces has proved useful in epidemiological studies of disease caused by bacteria of the genera *Staphylococcus* [5, 6], *Neisseria* [7–9], *Haemophilus* [10], *Campylobacter* [11] and *Streptococcus* [12]. Lectin typing is a simple and economical procedure suitable for use in non-specialist laboratories, either as an adjunct to

serogrouping or, where appropriate, as a sole typing scheme [11].

This study reports the patterns of agglutination of 77 clinical isolates of MRSA by 32 commercially available lectins. The bacterial suspensions were not pre-treated, as the aim was to investigate the binding of lectins to bacteria whose surface structure had not been modified. All isolates were tested after culture in Columbia blood agar, trypticase-soy agar and Chapman Stone agar, to investigate whether culture medium affects subsequent lectin binding.

Materials and methods

Lectins

The lectins used (Table 1) were selected on the basis of their nominal specificities to cover the widest possible range of sugars. All are commercially available (Sigma). Before use, the lectins were resuspended at 1 mg/ml in phosphate-buffered saline (PBS), pH 7.2–7.4, and stored at –20°C in 500-µl Eppendorf tubes.

Bacteria

A total of 77 MRSA isolates was provided by the Microbiology Laboratory of the Hospital de Conxo (Complejo Hospitalario Universitario de Santiago de Compostela, Galicia, Northwest Spain) between 1990 and 1996. Isolates were from clinical samples from the intensive care unit (23%), internal medicine (20%),

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Table 1. Panel of lectins

Lectin	Abbreviation	Common name	Sugar specificity
<i>Anguilla anguilla</i>	AA	Fresh-water eel	α -L-fuc
<i>Arachis hypogaea</i>	PN	Peanut	b-D-gal(1-3)-D-galNAc
<i>Bauhinia purpurea</i>	BP	Camel's foot tree	O- β -D-gal(1-3)-D-galNAc
<i>Canavalia ensiformis</i>	Con A	Jack bean	α -D-man, α -D-glc
<i>Codium fragile</i>	CF	Green marine algae	D-galNAc
<i>Cytisus scoparius</i>	CS	Scotch broom	galNAc, gal
<i>Datura stramonium</i>	DS	Jimson weed	(D-glcNAc) ₂
<i>Dolichus biflorus</i>	DB	Horse gram	α -D-galNAc
<i>Erythrina cristagalli</i>	EC	Coral tree	β -gal(1-4)glcNAc
<i>Euonymus europaeus</i>	EE	Spindle tree	α -gal(1-3)-D-gal
<i>Galanthus nivalis</i>	GN	Snowdrop bulb	non-reduc. α -man
<i>Glycine max</i>	SB	Soybean	D-galNAc
<i>Helix pomatia</i>	HP	Edible snail	D-galNAc
<i>Lens culinaris</i>	LC	Lentil	α -D-man
<i>Limulus polyphemus</i>	LP	Horseshoe crab	NeuNAc, D-glcNAc, D-galNAc
<i>Lycopersicon esculentum</i>	LE	Tomato	(D-glcNAc) ₃
<i>Maclura pomifera</i>	MP	Osage orange	α -D-gal, α -D-galNAc
<i>Narcissus pseudonarcissus</i>	NP	Daffodil	α -D-man
<i>Phaseolus limensis</i>	LB	Lima bean	D-galNAc
<i>Phaseolus vulgaris</i>	PHA	Red kidney bean	Oligosaccharide
<i>Phytolacca americana</i>	PWM	Pokeweed	(D-glcNAc) ₃
<i>Pisum sativum</i>	PS	Garden pea	α -D-man
<i>Ptilota plumosa</i>	PP	Red marine algae	α -D-gal
<i>Sambucus nigra</i>	SN	Elder	α -NeuNAc-(2-6) gal/galNAc
<i>Solanum tuberosum</i>	ST	Potato	(D-glcNAc) ₃
<i>Sophora japonica</i>	SJ	Pagoda tree	β -galNAc
<i>Tetragonolobus purpurea</i>	TP	Asparagus pea	α -L-fuc
<i>Triticum vulgaris</i>	WGA	Wheat-germ	(D-glcNAc) ₂ , NeuNAc
<i>Ulex europaeus</i> UEA I	UEA I	Gorse, furze	α -L-fuc
<i>Ulex europaeus</i> UEA II	UEA II	Gorse, furze	(D-glcNAc) ₂
<i>Vicia faba</i>	VF	Fava bean	D-glc, D-man
<i>Vicia sativa</i>	VS	–	D-glc, D-Man

surgery (13%), dermatology (11%) or other services (15%).

After incubation for 24 h at 37°C on Columbia blood agar, trypticase-soy agar and Chapman Stone agar, cells were harvested and washed three times by centrifugation in PBS at 1000 rpm for 10 min. Cell suspensions in PBS were adjusted to obtain an A₅₅₀ of 1.25 in a DMS Varian Spectrophotometer.

Antibiotic susceptibility testing

The isolates were tested for methicillin resistance by agar diffusion tests on Mueller Hinton Agar with 1- μ g oxacillin disks (Oxoid). Isolates were also assayed with a commercial oxacillin agar screen (BBL oxacillin screen agar; Becton Dickinson, Cockeysville, MD, USA). In all tests, the inoculum size was equivalent to a 0.5 McFarland standard and incubation was for 24 h at 35°C.

Agglutination assay

Assays were performed in 12-well glass plates (13 $\times$ 9 $\times$ 0.6 cm), by adding 15 μ l of lectin to 15 μ l of bacterial suspension in each well and shaking gently for 30 s. In each series a negative control was run in one well by adding 15 μ l of PBS instead of lectin to the bacterial suspension. Agglutination was assessed by microscopic examination.

Results

In the present study, cell suspensions were not pre-treated, as the aim was to investigate the binding of lectins to MRSA whose surface structure had not been modified. When trypsin or other proteolytic enzymes partially hydrolyse proteoglycans, some carbohydrate residues may become exposed and thus available for binding; equally, however, other residues may be destroyed or modified. Furthermore, pre-treatment increases the duration of the assay [12].

Of the 77 MRSA isolates tested, 16 (20.7%) were auto-agglutinating and thus could not be lectin-typed and 12 (15.5%) were not agglutinated by any of the lectins tested. The agglutination patterns observed for the remaining 49 isolates are listed for each culture medium in Table 2. Regardless of culture medium, the most frequent pattern was no agglutination by any of the lectins used (27.8% of isolates following culture in Columbia blood agar; 37.7% of isolates following culture in trypticase-soy agar; 42.6% of isolates following culture in Chapman Stone agar).

Of the bacteria cultured on Columbia blood agar, 21 (65.6%) of the 32 lectins tested agglutinated at least one isolate. The lectins agglutinating most isolates (for abbreviations see Table 1) were ConA (36 of 61 isolates, 59.0%), HP (29 isolates, 47.5%), PS (15 isolates, 24.5%) and LC (10 isolates, 16.3%). A total of 30 agglutination patterns was observed, the most

Table 2. Agglutination patterns

Date	Unit	Isolate No.	Agglutinating lectins after growth on		
			Columbia blood agar	Trypticase-soy agar	Chapman Stone agar
March 93	Internal medicine	1	Con A, HP, PS, LC, LP, CF, AA, LE	Con A, HP	Con A, HP, AA, PS
Oct. 90	Surgery	2	Con A, HP, PS, LC, WGA, CF, DS	Con A, HP, WGA, AA, CF, VF	Con A, HP, WGA, CF, EC, DS, VF
Oct. 96	Surgery	3	Con A, HP, PS, LC, WGA, CF	Con A, HP, PS, WGA, AA, LC	Con A, HP, AA, WGA, LC
March 93	ICU	4	Con A, HP, LC, WGA, CF, AA	Con A, HP, WGA, AA, CF	Con A, HP, AA, WGA, LC, LE, GN
Jan. 93	Dermatology	5	Con A, HP, PS, LC, AA	Con A, HP, PS	HP
Oct. 96	Surgery	6	Con A, HP, PS, LC, AA	Con A, HP, PS	HP, PS
June 92	Surgery	7	Con A, HP, LP, SN, VF	Con A, HP	Con A, HP, LP
Aug. 95	ICU	8	Con A, HP, WGA, CF, PN	Con A, HP, WGA, CF	Con A, HP, AA, WGA, CF, PN
July 93	ICU	9*	Con A, HP, WGA, AA, EC	Con A, HP, WGA, AA, EC	Con A, HP, AA, WGA, EC
Jan. 92	Internal medicine	10	PS, NP, SB, ST, VS	SB	HP, WGA, PHA, SB
Jan. 93	ICU	11	Con A, HP, PS, LC	HP	HP, ST
Aug. 96	Surgery	12+	Con A, HP, PS, LC	Con A, HP	Con A, HP
Jan. 93	ICU	13	Con A, HP, PS, LP	Con A, HP, PS	Con A, LP, PS
July 92	Dermatology	14*	Con A, HP, LP, EE	Con A, HP, LP, EE	Con A, HP, LP, EE
Dec. 95	Staff	15	Con A, HP, LP, NP	Con A, HP, LP	Con A, HP, LP, AA, LC, LE
Oct. 93	Surgery	16	Con A, HP, WGA, EE	Con A, HP, WGA	Con A, WGA
Dec. 95	Staff	17	Con A, HP, DS, LE	Con A, HP, LE	Con A, HP, AA, LE, TP
Oct. 92	ICU	18+	Con A, HP, PS	Con A, HP, PS	Con A, HP
Jan. 93	ICU	19	Con A, HP, PS	HP, PS	PS
March 93	ICU	20+	Con A, HP, PS	ConA, HP, PS	HP, PS
March 96	ICU	21	Con A, HP, LC	LP	PS, MP
July 93	Internal medicine	22+	Con A, HP, EE	Con A, HP, EE	EE
March 93	Internal medicine	23	Con A, LP, SN	No agglutination	LP
Nov. 92	Internal medicine	24+	Con A, HP	HP	HP
Nov. 92	Dermatology	25	Con A, HP	No agglutination	No agglutination
March 93	Internal medicine	26	Con A, HP	HP	No agglutination
March 93	ICU	27	Con A, HP	Con A, HP, WGA	HP, LP, AA
March 95	ICU	28*	Con A, HP	Con A, HP	Con A, HP
Aug. 95	Surgery	29	Con A, HP	Con A, HP, LC	Con A, LC
Nov. 92	Dermatology	30	Con A, PS	No agglutination	No agglutination
Oct. 92	ICU	31+	Con A, LP	Con A	Con A, LP
Oct. 92	ICU	32*	Con A, LP	Con A, LP	Con A, LP
Feb. 93	Internal medicine	33	HP, PS	No agglutination	HP
May 92	Urology	34	HP, EE	HP	No agglutination
Jan. 95	ICU	35	LC, DB	HP, LC	HP, LC, CF, MP, PP, DB
July 93	ICU	36	Con A	Con A, SJ, UEAI	Con A, LC
Nov. 95	Internal medicine	37+	Con A	Con A, HP	Con A
April 96	Internal medicine	38	Con A	No agglutination	No agglutination
June 96	ICU	39	Con A	No agglutination	No agglutination
June 96	ICU	40	Con A	No agglutination	No agglutination
March 93	ICU	41	PS	No agglutination	No agglutination
July 96	Oncology	42*	LP	LP	LP
Nov. 96	Dermatology	43	PN	No agglutination	No agglutination
April 92	Internal medicine	44	MP	No agglutination	No agglutination
Jan. 92	Internal medicine	45	No agglutination	PS	No agglutination
June 96	Dermatology	46	No agglutination	LP	No agglutination
Jan. 90	Surgery	47	No agglutination	EE	No agglutination
Aug. 95	Surgery	48	No agglutination	MP	No agglutination
Nov. 93	ICU	49	No agglutination	No agglutination	UEAI

*Isolates agglutinated by the same set of lectins independently of medium.

+Isolates agglutinated by the same set of lectins after culture in two of the three media.

frequent being agglutination by both ConA and HP (6 isolates, 9.8%).

With bacteria cultured on trypticase-soy agar, 16 (50%) of the 32 lectins tested agglutinated at least one isolate. The lectins agglutinating most isolates were HP (28 of 61 isolates, 45.9%), ConA (25 isolates, 40.9%), PS (8 isolates, 13.1%) and WGA (7 isolates, 11.4%). A total of 24 agglutination patterns was observed, the most frequent being (i) agglutination by both ConA and HP (5 isolates, 8.2%) and (ii) agglutination by ConA, HP and PS (5 isolates, 8.2%).

Of the bacteria cultured on Chapman Stone agar, 23 (71.8%) of the 32 lectins tested agglutinated at least one isolate. The lectins agglutinating most isolates were HP (22 of 61 isolates, 45.9%), ConA (20 isolates, 32.7%), LP (9 isolates, 14.7%) and AA (8 isolates, 13.1%). A total of 27 agglutination patterns was observed, the most frequent being (i) agglutination by HP alone (3 isolates, 4.9%) and (ii) agglutination by HP and ConA (3 isolates, 4.9%).

Regardless of culture medium, the lectins that bound most isolates were ConA (specific for α -D-mannose and α -D-glucose) and HP (specific for N-acetyl-D-

galactose). For a given isolate/medium/lectin combination, repeated assays gave consistent results (data not shown). Table 2 summarises details of the agglutination patterns observed for each isolate after culture in each of the three media, considering only the 49 isolates that were agglutinated in at least one assay (i.e., after culture in at least one medium, by at least one lectin). Only five isolates (nos. 9, 14, 28, 32 and 42) were agglutinated by the same lectins regardless of culture medium, and seven isolates (nos. 12, 18, 20, 22, 24, 31 and 37) were agglutinated by the same set of lectins after culture on two of the three media.

Discussion

Typing of MRSA strains is necessary for thorough epidemiological investigations of sources and modes of spread of these strains in hospitals and to design appropriate control measures [13]. Traditional typing methods, such as phage typing and determination of antimicrobial agent resistance profiles, often suffer from insufficient reproducibility, limited discriminatory power, or poor specimen typability, and a number of MRSA strains are not typable with routine phage panels, although use of supplementary phage panels may be helpful [14–19]. The variability inherent in discriminating among strains by phenotypic methods has stimulated interest in DNA-based typing systems [20–23]; but some MRSA strains lack plasmids and are therefore non-typable by plasmid profiling. However, molecular biology methods are costly, time-consuming and technically demanding, and they require inter-laboratory standardisation [24]. Currently, there is no single definitive typing system for distinguishing between individual strains of MRSA [25].

The ideal typing system should be rapid, inexpensive, technically simple and readily available. All these conditions are fulfilled by the lectins; moreover they are extremely sensitive to subtle structural differences among bacterial isolates and lectin typing allows differentiation of a greater number of MRSA groups than other typing systems [26].

As shown by the isolation dates (Table 2), strains sharing the same set of lectins were isolated during very short periods (isolates 24 and 25; 31 and 32; 39 and 40), with a high probability of being identical, and were also isolated after long periods of time (isolates 5 and 6; 11 and 12; 18, 19 and 20; 25 and 26; 36, 37 and 38), which may indicate periodic outbreaks of hospital endemic clones. Molecular genetic methods are required to establish the epidemiological origin of these strains; however, the results of the present study indicate that lectin typing may constitute a good first approach to the epidemiology of MRSA, as it can be performed rapidly in the routine of clinical laboratories.

Jarlov *et al.* (1993) identified a series of 'lectin types' when they examined MRSA isolates from Denmark, the UK and Singapore [26]. However, they did not specify the culture medium used. This is of critical importance in view of the present results, which indicate that the lectins that bind to a given MRSA isolate vary widely depending on the medium on which the isolate was cultured. This variation may be attributable to variation in the composition of different media, which may in turn affect the expression of sugar residues on the bacterial cell surface.

These results strongly suggest a need for researchers to specify the culture medium used in studies of this type, and ideally to agree on a single standard medium to be used for lectin typing of a given bacterium (in this case MRSA). As we have remarked previously [12], practising clinical microbiologists will reap the full benefits of lectin typing only when researchers have agreed on some degree of methodological standardisation. In the absence of larger-scale studies, we tentatively propose Columbia blood agar as the standard medium for lectin typing of MRSA, as it gave the largest number of binding patterns in the present study.

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