Clinical distribution and antibiotic sensitivities of staphylococcal strains isolated over an eight-month period

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SUMMARY A total of 842 staphylococci isolated from clinical material over an eight-month period and regarded as probable pathogens were identified according to lyogroup. Almost half the isolates belonged to lyogroups other than lyogroup I (*Staphylococcus aureus*), suggesting that coagulase-negative staphylococci are increasingly involved in human infections. All isolates were tested for sensitivity to 12 antibiotics. A greater resistance was observed in non-lyogroup I isolates, which again suggests a pathogenic significance of coagulase-negative staphylococci. Only lyogroup I strains, however, were obtained more frequently from clinical isolates than from healthy human skin. The distribution of the isolates in each lyogroup according to their clinical source is reported.

The significance and frequency of isolation of the different staphylococcal species from clinical sources has not been fully investigated to date. Only some specific aspects have been studied, such as the association of coagulase-negative staphylococci with urinary tract infections.¹⁻⁹ The lack of more comprehensive studies makes it difficult to evaluate the pathogenicity of the different staphylococcal species currently recognised.

In our laboratory, staphylococci are usually identified according to their lytic activity patterns. In previous studies, the type of lytic activity displayed by staphylococcal strains has been shown to have a high discriminatory power and taxonomic value within this bacterial genus.¹⁰⁻¹⁴ Six 'lyogroups' of human staphylococci identified according to their lytic activity patterns correlated well with human staphylococcal species recognised and identified according to wholly different criteria.^{11 12} Given such close relationships, it makes no substantial difference whether clinical staphylococcal isolates are identified according to lyogroup or to species. Both procedures have a similar aim and provide a similar amount of diagnostic information.

In this study lyogroup identification was performed according to a simplified system; its advantages with respect to other methods designed for routine identification of staphylococci have been shown in a comparative investigation.¹⁵

Material and methods

BACTERIAL STRAINS

A total of 842 staphylococci, all Gram-positive and catalase-positive cocci and belonging to the genus staphylococcus,^{15 16} were isolated from a variety of clinical specimens processed in our Institute's diagnostic laboratory over an eight-month period. All specimens were from inpatients in various clinical departments of the University of Genoa Medical School. Many more staphylococci than the number reported above were isolated during the eight-month study period, but those isolates thought not to have clinical relevance were disregarded. Several parameters were considered in evaluating whether the isolates were to be regarded as clinically relevant saprophytic organisms or contaminants. Such parameters included the nature of the specimen, the procedure of specimen collection, the quantitative amount of colony-forming units yielded by the culture, the association (regarded both quantitatively and qualitatively) with other organisms in mixed cultures, relation to the patient's condition and symptoms, and repeated isolation from consecutive specimens.

ROUTINE LYOGROUP IDENTIFICATION

A simplified lyogroup identification system was used.¹⁵ Preparation and inoculation of the test media, determination of lytic activity pattern and phosphatase activity, and the resulting lyogroup identi-

fication were carried out according to previously described procedures.¹⁵⁻¹⁷

ANTIBIOTIC SENSITIVITY TESTING

The sensitivity of the isolates to 12 antimicrobial agents was determined using the standard agar diffusion method. Plates of DST agar (Oxoid) supplemented with 5% defibrinated horse blood were inoculated with suitably diluted broth cultures in order to obtain a just confluent growth after incubation. Antibiotic-impregnated discs were applied to the surface of the inoculated plates using a 12-disc dispenser. After overnight incubation at 37°C the plates were examined, and the diameters of the inhibition zones were measured to determine antibiotic sensitivities. When intermediate zone sizes were observed, that is, halfway between sensitive and resistant, the strains were recorded as resistant. The 12 antibiotics tested and the respective contents of the discs used were as follows: penicillin G (10 units), oxacillin (1 µg), methicillin (5 µg), cephalothin (30 µg), fosfomycin (50 µg), tetracycline (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), lincomycin (2 µg), gentamicin (10 µg), nitrofurantoin (100 µg), and co-trimoxazole (sulphamethoxazole 23.75 µg + trimethoprim 1.25 µg). Discs were obtained from BBL, Cockeysville, Md, USA, except for fosfomycin, which was obtained from Italchemi SpA, Parma, Italy.

Results

All the clinical isolates of staphylococcus were

identified using the simplified lyogroup system.¹⁵ With the exception of seven strains whose lyogroup could not be determined, all other isolates were identified as belonging to one of the six known lyogroups of human staphylococci. The total number and percentage of staphylococci in each lyogroup are shown in Table 1 together with previously established relationships between staphylococcal lyogroups and species.¹²⁻¹⁵ Over half of the isolates (52%) were members of lyogroup I. Among the other staphylococci, strains of lyogroup V (25%) and lyogroup VI (13%) predominated. There were fewer isolates in lyogroup IV (6%), lyogroup II (2%), and lyogroup III (1%).

Table 1 Lyogroup distribution of 842 clinical isolates of staphylococcus

Lyogroup	Related species	No. of isolates	%
I	<i>Staph. aureus</i>	436	52
II	<i>Staph. simulans</i>	16	2
III	<i>Staph. capitis</i>	11	1
IV	<i>Staph. saprophyticus</i> , <i>Staph. cohnii</i> , <i>Staph. xylosus</i>	48	6
V	<i>Staph. epidermidis</i>	214	25
VI	<i>Staph. hominis</i> , <i>Staph. haemolyticus</i> , <i>Staph. warneri</i>	110	13
Unidentified		7	1

In Table 2 the isolates are listed according to lyogroup and clinical source. The largest number of clinically relevant staphylococci were obtained from wounds and abscesses (23%), urine (12%), throat

Table 2 Distribution of 842 staphylococcal isolates according to lyogroup and clinical source

Clinical source	Lyogroup							Total (842)
	I (436)	II (16)	III (11)	IV (48)	V (214)	VI (110)	U (7)	
Blood	6*			2	18	2		28
Urine	13	6	2	20	35	25	1	102
Urethral, prostatic secretion	7		1	4	14	12		38
Semen	1				9	3		13
Vagina	7			3	15	6	1	32
Stool, rectal swab	15			1	7	4		27
Bile	8		1	2	9		1	21
Sputum, bronchial or tracheal aspirate	59	2	1	5	8	7	1	83
Throat, oral cavity	83			1	6	10		100
Nose	16				10	3		29
Ear	18		1		8			27
Eye	16		2	1	5	2		26
Cerebrospinal fluid	4				2			6
Joint, pleural, peritoneal fluid	13		1		4	5		23
Osteomyelitis pus	14	1			1			16
Wounds, abscesses	132	5	2	5	35	16	2	197
Skin	14	2		2	10	8	1	37
Indwelling artificial devices (catheters, valves, etc)	1				11	1		13
Other sources	9			2	7	6		24

U = unidentified.

Numbers in parentheses indicate number of isolates in each group.

*Data are given as numbers of isolates.

and oral swabs (12%), and respiratory secretions (10%).

The patterns of antimicrobial resistance of the strains of each lyogroup to 12 antibiotics are shown in Table 3. A total of 11% of all isolates were sensitive to all antibiotics tested; five (1%) were resistant to gentamicin, 16 (2%) to cephalothin, and 24 (3%) to nitrofurantoin. Increasing rates of resistance were obtained for chloramphenicol (7%), co-trimoxazole (10%), lincomycin (13%), erythromycin (14%), methicillin (14%), oxacillin (22%), fosfomycin (26%), and tetracycline (31%). The highest rate of resistance was to penicillin (65%).

Discussion

Lyogroup I (*Staphylococcus aureus*) was found to predominate among the clinical isolates of staphylococcus, but not to the extent that one might expect in view of the conventional idea that coagulase-negative staphylococci are less pathogenic. Although the actual clinical relevance of a staphylococcal isolate is sometimes difficult to substantiate, even when rigorous criteria are applied, the fact that almost half of the staphylococci isolated from clinical material over an eight-month period and regarded as likely pathogens were not *Staph. aureus* is one crucial aspect of this study. Similar ratios of *Staph. aureus* to non-*Staph. aureus* clinical isolates have been reported in other surveys.^{18 19} It should be noted, however, that, with the exception of lyogroup I, the distribution among the different lyogroups of the clinical staphylococcal isolates was similar to that of the average staphylococcal flora of healthy human skin^{20 21} (and unpublished results). This indicates that *Staph. aureus* has a special ability to overcome host defences, whereas other staphylococci

are less able to do so and do not show particular differences from one lyogroup (or species) to another.

As for lyogroup distribution according to clinical material, the prevalence of lyogroup I from most sources and of lyogroup V from blood, genital secretions, or indwelling artificial devices is in agreement with what has been reported for *Staph. aureus* and *Staphylococcus epidermidis* in previous surveys.^{18 19 22-24} Our findings regarding urinary isolates, on the other hand, differ from other reports. We did not find the predominance of novobiocin-resistant staphylococci (*Staphylococcus saprophyticus*, above all) reported by several authors.^{5-7 25-29} In our study, lyogroup IV strains—those overlapping with novobiocin-resistant staphylococci—were isolated in larger numbers from urine than from other specimens, but their frequency among urinary isolates was lower than that of lyogroup V and lyogroup VI strains. It should be noted also that in another recent report⁹ *Staph. saprophyticus* strains (or more generally novobiocin-resistant staphylococci) were not the most common cause of staphylococcal infections of the urinary tract. This is probably due to the fact that the specimens examined in our study were from inpatients of both sexes and all ages, whereas *Staph. saprophyticus* has been described as a cause of urinary infections virtually confined to young female outpatients.^{5-7 26}

Our findings regarding the antibiotic sensitivity of clinical staphylococcal isolates agree with the current view that coagulase-negative staphylococci tend to become more resistant than their coagulase-positive counterparts.^{9 30 31} This stresses the pathological importance of coagulase-negative staphylococci and also suggests that their involvement in human infections is likely to increase. In our investigation, for 10 of the antibiotics tested the

Table 3 Antibiotic resistance of 842 clinical staphylococcal isolates identified according to lyogroup

Antimicrobial agents	Lyogroup												Total non-lyogroup I isolates (406)		Total isolates (842)			
	I (436)		II (16)		III (11)		IV (48)		V (214)		VI (110)						U (7)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Penicillin G	314	72	8	50	3	27	8	17	147	69	63	57	2	29	231	57	545	65
Oxacillin	78	18	5	31	1	9	26	54	43	20	32	29	1	14	108	27	186	22
Methicillin	27	6	0	0	0	0	15	31	56	26	24	22	0	0	95	23	122	14
Cephalothin	0	0	0	0	0	0	1	2	4	2	11	10	0	0	16	4	16	2
Fosfomycin	70	16	0	0	8	73	32	67	36	17	73	66	2	29	151	37	221	26
Tetracycline	81	19	3	19	3	27	10	21	120	56	40	36	2	29	178	44	259	31
Chloramphenicol	9	2	1	6	0	0	3	6	23	11	22	20	0	0	49	12	58	7
Erythromycin	35	8	4	25	0	0	7	15	38	18	30	27	0	0	79	19	114	14
Lincomycin	40	9	5	31	0	0	7	15	32	15	24	22	1	14	69	17	109	13
Gentamicin	0	0	0	0	0	0	0	0	0	0	5	5	0	0	5	1	5	1
Nitrofurantoin	21	5	0	0	0	0	0	0	0	0	3	3	0	0	3	1	24	3
Co-trimoxazole	31	7	1	6	0	0	0	0	39	18	12	11	0	0	52	13	83	10

U = unidentified.

Numbers in parentheses indicate number of isolates in each group.

incidence of resistance was higher among non-lyogroup I than among lyogroup I isolates, with increases from 1.5 times for oxacillin to 6 times for chloramphenicol. None of the isolates resistant to cephalothin or gentamicin was in lyogroup I. The incidence of resistance was higher among lyogroup I than among non-lyogroup I isolates for penicillin and nitrofurantoin only. All of the five gentamicin-resistant isolates encountered in this study belonged to lyogroup VI, and of the 24 nitrofurantoin-resistant isolates, 21 belonged to lyogroup I and three to lyogroup VI. A high overall antibiotic sensitivity was shown by lyogroup III, which agrees with what has been reported elsewhere for *Staph. capitis*.⁹ On the other hand, the greatest incidence of fosfomycin-resistance was found within this lyogroup. Lyogroups V and VI were, on the whole, the most resistant to antibiotics.

With regard to the open question whether an in-depth or limited discrimination within the genus *Staphylococcus* is preferable in clinical bacteriology,³² our findings suggest that a rather precise discrimination is desirable. Preliminary indications of certain lyogroup- (or species-) specific antimicrobial susceptibility patterns and associations with particular infection sites advise against *a priori* limiting the identification of staphylococcal species or lyogroups. A real knowledge of the epidemiology and pathological significance of each particular *Staphylococcus* species or lyogroup will be possible only if accurate identification of the isolate is the rule in diagnostic laboratories. Routinely performed species separation within other genera of pathogenic organisms is recommended for similar reasons. On the other hand, practical and rapid identification of staphylococci, such as that obtained with our simplified lyogroup method, can be performed routinely even in poorly equipped diagnostic laboratories.

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