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Bacteriophage Types and Antibiotic Susceptibility of *Staphylococcus aureus*

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In vitro tests of 132 strains of *Staphylococcus aureus*, among which were 38 (28.7%) heteroresistant strains, were performed with 14 commonly used antibiotics, including gentamicin and vancomycin. Heteroresistant strains were found predominantly, but not only, with group 3 phage-typable strains; no heteroresistant strain was found in out-patients. Gentamicin appeared as a uniformly effective agent at low concentrations against oxacillin-sensitive and -resistant strains. It is suggested that gentamicin should be compared to vancomycin in future clinical therapeutic trials in severe staphylococcal infections, especially in cases due to oxacillin-resistant strains.

The susceptibility of pathogenic strains of *Staphylococcus aureus* has been reviewed recently in several institutions (1, 13). It appears that the incidence of antibiotic resistance among hospital-isolated staphylococci is decreasing (1, 6); however, heteroresistant strains were poorly represented in these series. The sensitivity to antibiotics of these heteroresistant strains has been studied (2, 4), but their susceptibility to newer drugs such as gentamicin and minocycline, that are known to inhibit staphylococci in vitro, has not yet been fully investigated.

Heteroresistant strains of *S. aureus* may be important clinically and epidemiologically (7); they have been isolated predominantly in Europe until now, but it is possible that these strains will become more widespread elsewhere.

The present study was undertaken to obtain current data on the prevalence of various bacteriophage types and of resistance to antibiotics among *S. aureus* strains encountered at the Institut Jules Bordet, which is the clinical center for cancer therapy of the University of Brussels.

MATERIALS AND METHODS

The 132 strains of *Staphylococcus aureus* included in this survey were isolated in the Bacteriology Laboratory at the Institut Jules Bordet between 1 January 1970 and 31 December 1970. All strains that were isolated in pure or predominant cultures were included in this series, but only one isolate of the same bacteriophage pattern and antibiogram from each patient was studied.

The tube coagulase test and the test for detection of deoxyribonuclease activity (DNase test agar, BBL) were used for confirmation and always gave concordant results.

The sensitivity of these strains was tested routinely by the disc method by using Mueller Hinton agar (Difco) and commercial discs (produced by B-D Mérieux under BBL license) as recommended by Bauer et al. (3) with two slight modifications: discs impregnated with 2 μg of oxacillin were used, and a zone diameter of 11 mm was considered to indicate strains sensitive rather than intermediate to this antibiotic. Single colonies were transferred to Trypticase soy broth (BBL), incubated at 37 C overnight, and stored at 4 C until used for bacteriophage typing and antibiotic-sensitivity testing.

Bacteriophage typing was performed at the Institut Pasteur du Brabant with the basic set of 22 bacteriophages recommended by the International Subcommittee on Phage-Typing of Staphylococci plus phage 88. The number of bacteriophages in this study was limited to this basic set, and the bacteriophages were used in the "routine test dilution"; those strains that were untypable were further tested at 100 times the routine test dilution, according to the decision taken by the Subcommittee of the International Association of Microbiological Societies (IAMS) at its 5th meeting in 1970.

Quantitative tests for susceptibility to antibiotics were carried out with the inocula-replicating method of Steers at al. (12) with Mueller Hinton agar containing twofold dilutions of antibiotic. The cultures were grown in Trypticase soy broth and used undiluted after overnight incubation at 37 C. The antibiotics used were penicillin G, oxacillin, cephalothin, cephaloridine, chloramphenicol, tetracycline, minocycline, doxycycline, kanamycin, gentamicin, erythromycin, lincomycin, novobiocin, and vancomycin.

The detection of resistance to oxacillin was performed with oxacillin-impregnated discs (2 μg) and

5% NaCl-agar (Mueller Hinton agar). The plates were inoculated with streaks of overnight broth cultures of the test organisms, and the paper discs were applied to the inoculated areas. The plates were incubated at 30 C.

All of the strains of *S. aureus* included in this series were also inoculated into broth containing oxacillin. Two-tenths milliliter of an undiluted overnight culture of the test organism was inoculated into 1 ml of Trypticase soy broth containing 50 μg of oxacillin. Tubes were incubated at 37 C and examined after 48 hr for the presence of viable staphylococci.

RESULTS

Tests for oxacillin resistance. With the exception of one, all of the 132 strains studied here gave

TABLE 1. *Relationship between the site of isolation of Staphylococcus aureus and the incidence of oxacillin-resistant (OR) strains*

Site of isolation	No. of strains				
	Total no. of isolates	Out-patients		In-patients	
		No. isolated	OR	No. isolated	OR
Blood	7	0	0	7	0
Wounds	62	17	0	45	22
Respiratory tract	63	2	0	61	16
Total	132	19	0	113	38

TABLE 2. *Distribution of bacteriophage patterns and groups among 132 strains of Staphylococcus aureus isolated at the Institut Jules Bordet, Brussels*

Bacteriophage patterns	No. of strains				
	RTD[a]	RTD (100×)	Heteroresistant strains	Out-patients	Total
Group 1					
29/52, 29/52/80, 29/52/81	13	0	2	0	13
52/52A/80	6	0	3	0	6
80, 80/81	5	0	1	0	5
29	2	0	1	0	2
Others	2	2	1	0	4
			[8][b]	[0]	[30 (22.7%)]
Group 2					
3C	1	1	[0]	[0]	[2 (1.4%)]
Group 3					
47/53/54/75/77/83A	2	0	0	0	2
6/47/53/54/75/83A/88+	4	0	0	0	4
54/75/77/84/81+	2	0	0	0	2
29/6/47/54/75/81+	2	0	2	0	2
Others	9	6	9	2	15
			[11]	[2]	[25 (18.9%)]
Miscellaneous					
81	8	2	3	3	10
187	1	1	0	0	2
88	5	2	4	2	7
			[7]	[5]	[19 (14.4%)]
Mixed					
Group 1 + 3	3	2	3	1	5
Group 1 + 2 + 3	0	1	0	0	1
Group 1 + 3 + miscellaneous	0	1	0	1	1
Others	1	1	2	1	2
			[5]	[3]	[49 (6.8%)]
Nontypable			[7]	[9]	[47 (35.5%)]

[a] RTD, routine test dilution.
[b] Values in brackets represent totals for the group.

zones wider than 11 mm in diameter around oxacillin-impregnated discs, when tested by a standard routine method (3). On 5% salt-agar, 38 strains grew up to or within 1 mm of the oxacillin disc. These 38 strains were considered to contain organisms resistant to oxacillin; the frequency of recovery of oxacillin-resistant strains was thus 28.7%. Thus our data cast doubt on the reliability of routine laboratory methods to establish resistance of *S. aureus* to oxacillin. Since the only strain for which resistance to oxacillin has been detected by a routine method has been isolated from an empyrema complicating a bronchopleural fistula that developed in a patient after incomplete resection of a bronchial carcinoma and who had undergone, prior to the hospitalization in our hospital, a prolonged therapy with oxacillin, it may be assumed that this patient had also been infected originally with a heteroresistant strain.

Among those strains detected as oxacillin-resistant by the 5% salt-agar test, 21 were able to grow in Trypticase soy broth containing 50 μg of oxacillin per ml. The use of the oxacillin disc on 5% salt-agar at 30 C detected more strains resistant to oxacillin than the use of an oxacillin-containing medium (50 μg/ml). Similar conclusions have been reached by other investigators regarding the detection of methicillin resistance (8).

The relationship between the site of isolation and the recovery of heteroresistant strains is illustrated in Table 1. None of the resistant strains has been isolated from blood cultures; their incidence in wound cultures was 48.8% (22

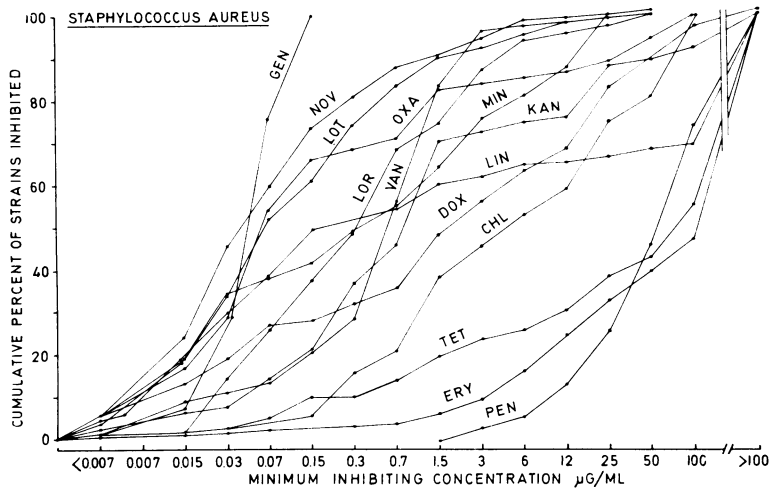

FIG. 1. *Antibiotic spectrum of 132 strains of Staphylococcus aureus.*

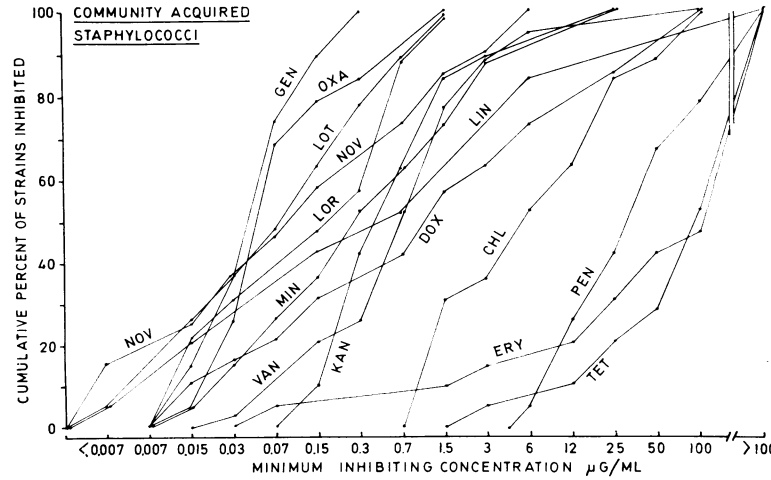

FIG. 2. *Antibiotic spectrum of staphylococci isolated from out-patients.*

strains) and 26.2% (16 strains) in cultures of the respiratory secretions. All of the heteroresistant strains were isolated from in-patients, and the incidence of heteroresistance was 33.6% in this group.

The clinical significance of the oxacillin-resistant staphylococci was of minor importance in this series. Most isolates from the respiratory tract were considered to represent bacterial colonization with no indication of antibiotic therapy. Most isolates from wounds responded satisfactorily to surgical drainage without administration of antibiotics. In all other cases, including the seven episodes of bacteremia, oxacillin was given and the infection was promptly controlled, with the exception of that which occurred in the patient whose strain was resistant to oxacillin by the routine test. The infection in that case responded well to the administration of vancomycin. Our data are in accordance with the conclusion reached by Chabbert and co-workers (7) that in most cases infections caused by heteroresistant strains are not resistant clinically to oxacillin. It might be suspected that therapy with semisynthetic penicillins or cephalosporins might become a serious problem only when the infection is caused by a majority of oxacillin-resistant staphylococci.

Bacteriophage typing. In this series, 47 strains (35.5%) could not be phage-typed. This is a higher figure than that reported from the United States (1) and from England (11). Among these strains, 7 (14%) were heteroresistant. Nine nontypable strains were isolated from out-patients. Group one included 30 strains, of which 8 (26%) were heteroresistant; this group was not represented in out-patients. Group three had a high frequency of heteroresistant strains: 11 (44%) out of 25 strains; only 2 strains belonging to the phage three group were isolated from out-patients. Bacteriophage types 81, 187, and 88 were represented by 19 strains, 7 (38%) of which were heteroresistant (Table 2).

Other reports have emphasized the frequency of heteroresistant strains in phage group three and among nontypable strains (1, 4). This was not verified in this series with respect to nontypable strains; moreover, the relationship between the pattern of lysis and the frequency of isolation of heteroresistant strains could not be established significantly. Heteroresistant strains were represented by 11 strains in group three and by 8, 7, 5, and 7 strains in groups one, miscellaneous, mixed, and nontypable, respectively. No significant relationship could be found between the pattern of bacteriophage lysis and the site of isolation, but groups one and two were not represented among outpatients and only two (8%) strains belonging to group three were found in out-patients. The relationship between bacteriophage types and susceptibility to antibiotics will be discussed below.

Susceptibility to antibiotics. The sensitivity of 132 strains of *S. aureus* to 14 commonly used antibiotics is illustrated graphically in Fig. 1. Of the accepted antistaphylococcal drugs, oxacillin and cephalothin were the most active antibiotics and minocycline was the most active of the three tetracyclines tested. None of these strains was inhibited by less than 1.5 μg of penicillin G per ml.

The median inhibitory concentrations (MIC) found in this series were approximately similar to those observed by Barrett and co-workers (1)

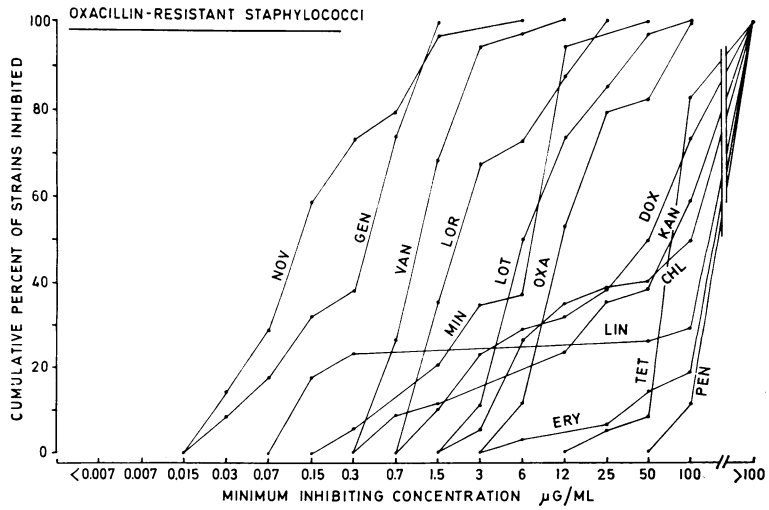

FIG. 3. *Antibiotic spectrum of oxacillin-resistant staphylococci.*

with the exception of erythromycin and tetracycline which were much less active here. Moreover, for all of the antibiotics we found a much more marked variation in the susceptibility of our strains. It has been reported that factors in media may be responsible for differences in MIC (13). Our results with gentamicin are similar to those reported by Hoeprich (9); it was the most active drug in our study, and 100% of the strains were inhibited by 0.15 μg of gentamicin per ml.

Staphylococci that have been isolated from out-patients exhibited a pattern of sensitivity to the various antibiotics tested similar to that described for the whole group (Fig. 2). On the

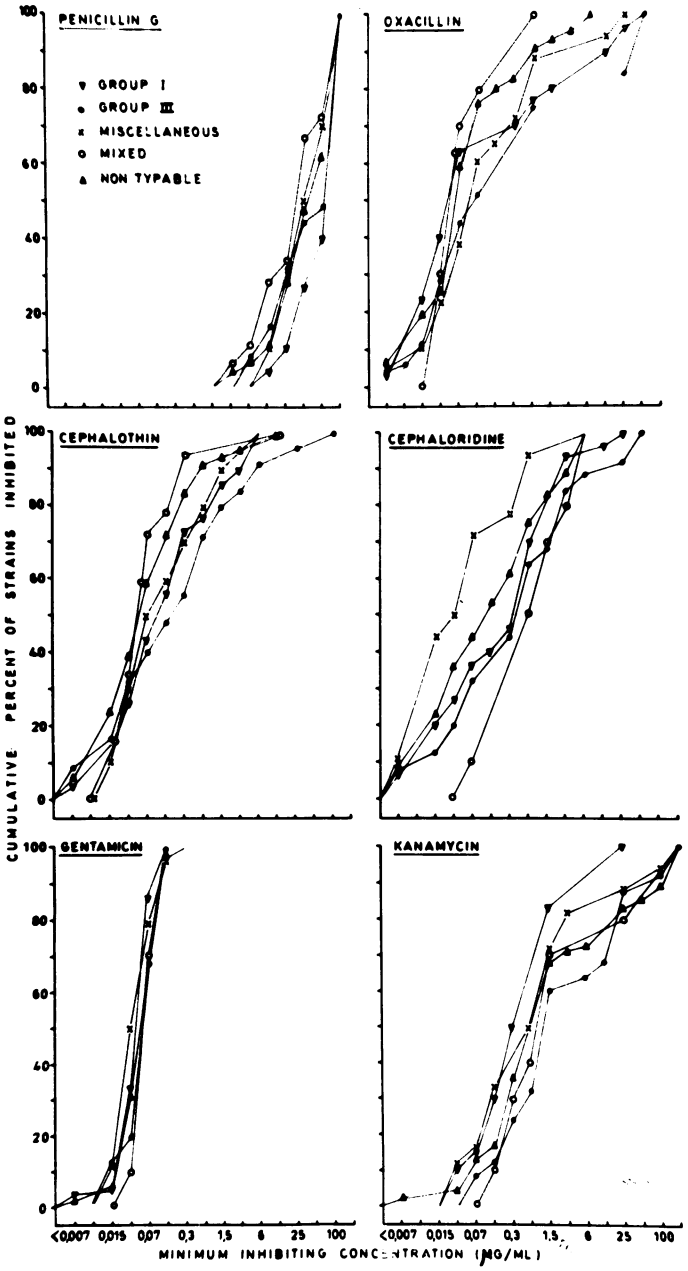

FIG. 4

FIG. 4, 5, and 6. *Relationship between results of bacteriophage typing and susceptibility to antibiotics.*

other hand, oxacillin-resistant staphylococci were much more resistant to the antibiotics used (Fig. 3). The bacteriostatic range for oxacillin against these strains extended from 3 μg/ml to 50 μg/ml and was quite similar to that of cephalothin, illustrating the cross-resistance between penicillinase-resistant penicillins and cephalosporins for these strains.

Novobiocin, gentamicin, and vancomycin were the most active drugs against oxacillin-resistant strains and inhibited 100% of them within a narrow range of concentrations which can be easily reached in the blood stream. Our data are in accordance with previous reports (2, 4) showing that novobiocin is highly effective in vitro against these strains and that vancomycin might well be the drug of choice; moreover, our studies suggest that gentamicin might prove to be a valuable therapy for infections caused by oxacillin-resistant staphylococci.

The relationship between the bacteriophage types and the susceptibility to antibiotics of the staphylococci studied in this series is illustrated in Fig. 4, 5, and 6. The median MIC values of penicillin G, oxacillin, novobiocin, minocycline, cephalothin, kanamycin and gentamicin were approximately the same, regardless of the bacteriophage type. On the other hand, the median MIC varied more widely for cephaloridine and vancomycin in the various bacteriophage groups. Strains belonging to group 3 and to groups 81, 187, and 88 were more resistant to these two antibiotics. However, these variations in susceptibility occurred within a range of concentrations that can

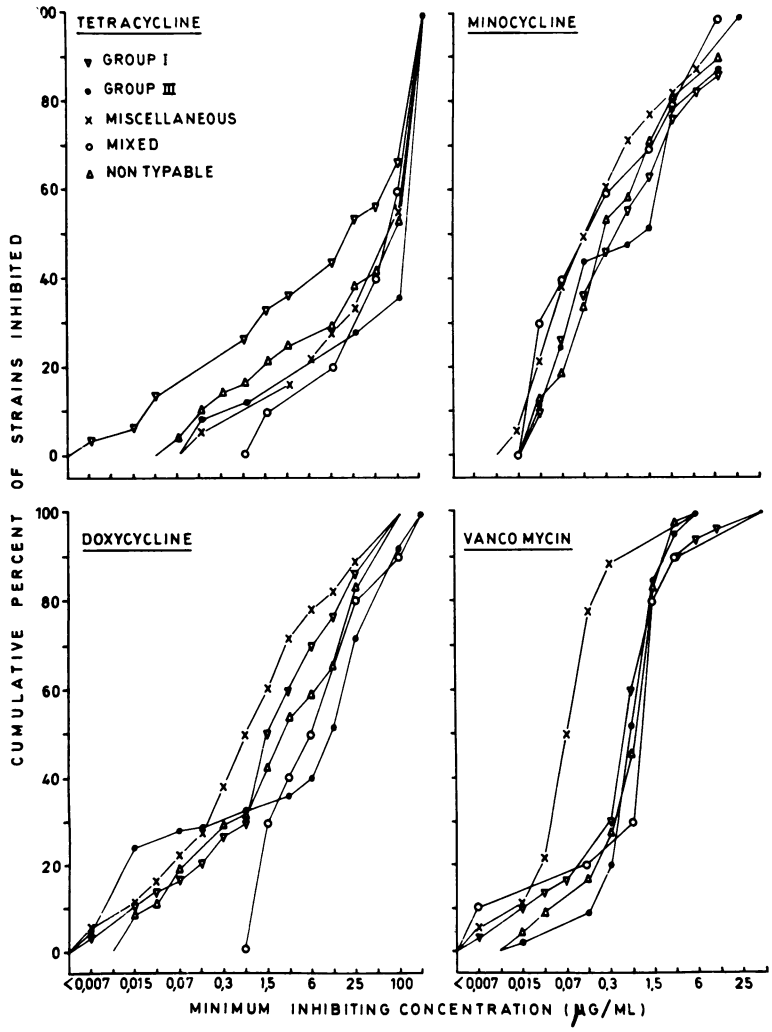

FIG. 5

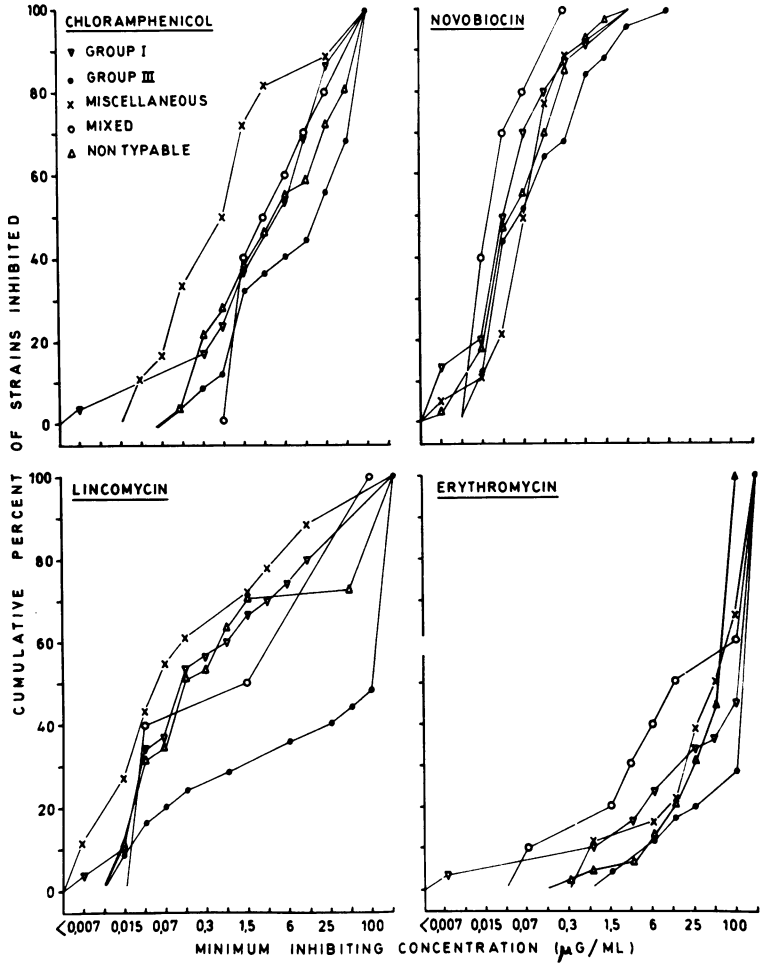

FIG. 6

be easily reached in the blood, and their clinical significance may be questioned. Staphylococci of group three were significantly more resistant to tetracycline, doxycycline, chloramphenicol, erythromycin, and lincomycin. The range of median MIC for these antibiotics covered a wide range of concentrations, including, as far as staphylococci of group three are concerned, levels that may be difficult to reach or maintain in the bloodstream.

DISCUSSION

The interest of the present study is that the susceptibility of a series of staphylococci containing an important proportion of heteroresistant strains has been tested for the commonly used antibiotics, including gentamicin for which antistaphylococcal activity has been reported (9).

The clinical significance of the heteroresistant strains might not be great. Chabbert and coworkers showed that higher rates of clinical and bacteriological failure may occur among patients infected with such staphylococci (7). However, our clinical results were uniformly good, regardless of the heteroresistant nature of the strains involved, with the exception of one case in whom oxacillin-resistant staphylococci were recovered at the time of admission to the hospital. Such clinical conditions are probably rare but may become a serious nosocomial problem in the future. Therefore, the study of antibiotic susceptibility of oxacillin-resistant strains may be helpful in orienting antimicrobial therapy when such strains are involved in sepsis.

Although most strains reported exhibited reactions with group three phages or could not be typed (4), our study clearly shows that heteroresistant strains may be found in most phage

groups. It has been reported that methicillin-resistant staphylococci are chiefly involved in hospital-acquired infections (2). In the present study, no heteroresistant strain has been isolated from out-patients, suggesting again that the selective pressure of antibiotics within the hospital may be the major contributing factor to the emergence of oxacillin-resistant staphylococci.

It has also been reported that vancomycin may be the best antimicrobial agent for treatment of infections due to staphylococci that are resistant to semisynthetic penicillinase-resistant penicillins (4). Our in vitro data are in accordance with these conclusions. However, it should be pointed out that gentamicin was, in this study, more active on both oxacillin-sensitive and oxacillin-resistant staphylococci.

Gentamicin has been used successfully in the treatment of serious staphylococcal infections (10). It seems, therefore, highly desirable that clinical experience of therapy of staphylococcal infections, especially of these infections caused by heteroresistant or oxacillin-resistant strains, with gentamicin be extended. Although administration of vancomycin frequently results in serious untoward effects, one may speculate that with gentamicin, which inhibits 100% of staphylococci at relatively low levels, toxicity would not be a major problem.

Another approach to therapy of infections caused by oxacillin-resistant staphylococci has been proposed by Bulger (5), who found in vitro synergism of kanamycin and cephalothin against such strains. However, some strains tested by Bulger grew in combinations of kanamycin and cephalothin. This suggests that aminoglycoside antibiotics may not be effective against certain members of these resistant strains.

Our study suggests that, in severe infection suspected to be caused by oxacillin-resistant staphylococci, vancomycin or gentamicin therapy should be considered. The clinical effectiveness of these regimens should be compared in the future.

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