NOTES

Antibiotic Susceptibility of Oxacillin-Resistant Staphylococci

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This study leads to the conclusion that gentamicin plus cephalothin or oxacillin is as bactericidal as vancomycin for oxacillin-resistant strains of *Staphylococcus aureus* in vitro. This finding suggests that these regimes might prove valuable in the therapy of infections caused by oxacillin-resistant staphylococci.

Oxacillin-resistant strains of *Staphylococcus aureus* may be important clinically and epidemiologically. In France, Chabbert and co-workers showed that higher rates of clinical and bacteriological failure of treatment occur among patients infected with such staphylococci (3). Vancomycin (1) or a combination of cephalothin and kanamycin (2) are considered by some to represent the drugs of choice for the treatment of severe infections caused by oxacillin-resistant strains. However, both forms of therapy are potentially toxic, and cephalothin plus kanamycin have not been shown effective against all strains tested.

Recent work from this laboratory (6) has shown that gentamicin was more active than vancomycin on a weight basis. This finding suggested that low, and thus relatively nontoxic, doses of gentamicin might represent an adequate therapy for infections caused by oxacillin-resistant staphylococci.

This study has been performed to investigate further the bactericidal action of gentamicin against oxacillin-resistant strains of *S. aureus* and to compare its action to that of vancomycin and of cephalothin plus kanamycin. It will be shown that gentamicin in combination with oxacillin or cephalothin is as effective as vancomycin against oxacillin-resistant staphylococci.

Twenty-nine strains of oxacillin-resistant staphylococci were used in this study. All had been isolated consecutively from clinical material at the Institut Jules Bordet. The resistance to oxacillin of these staphylococci was tested with oxacillin-impregnated discs (2 µg; purchased from B. D. Mérielux; manufactured under BBL license) and 5% NaCl agar (Müller-Hinton agar, Difco). 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 the strains grew up to or within 1 mm of the oxacillin disc.

Tests of bactericidal action were performed simultaneously for all antibiotics or antibiotic combinations tested against a given strain. Overnight cultures of the test organism in Trypticase Soy Broth (Difco) were adjusted to a final concentration of 10^7 viable organisms per ml by comparing the optical turbidity of the culture to be tested with a barium chloride standard. They were exposed to the following antimicrobial regimens: oxacillin, 50 µg/ml; cephalothin, 50 µg/ml; vancomycin, 20 µg/ml; kanamycin, 20 µg/ml; gentamicin, 5 µg/ml; cephalothin (50 µg/ml) plus kanamycin (20 µg/ml); cephalothin (50 µg/ml) plus gentamicin (5 µg/ml); and oxacillin (50 µg/ml) plus gentamicin (5 µg/ml). These bacterial suspensions were incubated at 37 C. At 1, 2, 4, 18, and 36 hr after the exposure to the antibiotics, samples were plated on Müller-Hinton agar plates, and bacterial counts were performed. For each strain studied, controls were examined by the same technique.

The bacteriophage types of the strains of *S. aureus* used in this study are shown in Table 1. In our study of a limited number of strains, no relationship between the bacteriophage type and the pattern of drug susceptibility has been found. However, in a previous study (6), we were not able to detect any significant relationship between the bacteriophage type of strains of *S. aureus* and their susceptibility to oxacillin, cephalothin, kanamycin, gentamicin, or vancomycin. All the strains tested here were able to

TABLE 1. Frequency of bactericidal action of antibiotics alone or in combination on 29 strains of oxacillin-resistant staphylococci

Strains	Phage type	Antibiotic(s) tested ^a							
		OXA	CEP	KAN	VAN	GEN	CEP + KAN	CEP + GEN	OXA + GEN
1	NT ^b	— ^c	—	—	+	+	—	+	+
2	NT	—	—	—	+	+	—	+	+
3	NT	—	—	—	—	—	+	+	+
4	NT	—	—	—	+	—	+	+	+
5	NT	—	—	—	+	+	+	+	+
6	NT	—	—	—	+	—	+	+	+
7	NT	—	—	—	+	+	+	+	+
8	I	—	—	—	+	+	+	+	+
9	I	—	—	—	+	+	+	+	+
10	I	—	—	—	—	+	+	+	+
11	I	—	—	—	+	—	+	+	+
12	III	—	—	—	+	+	—	+	+
13	III	—	—	—	+	—	+	+	+
14	III	—	—	—	+	+	+	+	+
15	III	—	—	—	+	—	+	—	+
16	III	—	—	—	+	—	—	+	—
17	III	—	—	—	+	+	+	+	+
18	III	—	—	—	+	—	+	+	—
19	III	—	—	—	+	+	+	+	+
20	I + III	—	—	—	—	—	+	+	+
21	I + III	—	—	—	+	—	—	+	+
22	I + III	— ^c	—	—	+	+	+	+	+
23	81	—	—	—	+	+	+	+	+
24	81	—	—	—	+	+	+	+	+
25	81	—	—	—	+	+	+	+	+
26	88	—	—	—	+	+	—	+	+
27	88	—	—	—	+	+	+	+	+
28	78/89	—	—	—	+	+	—	+	+
29	29/77/81	—	—	—	+	+	+	+	+

^a Total number of strains on which no bactericidal effect could be obtained: 29 with oxacillin (OXA), 29 with cephalothin (CEP), 29 with kanamycin (KAN), 3 with vancomycin (VAN), 10 with gentamicin (GEN), 7 with CEP plus KAN, 1 with CEP plus GEN, and 2 with OXA plus GEN.

^b NT, Not typable.

^c —, No bactericidal action; +, bactericidal action.

grow in the presence of relatively high doses of oxacillin, cephalothin, and kanamycin (Fig. 1). Some bactericidal action was noticed with kanamycin during the first 4 hr of the study. However, none of the 29 strains tested was killed after 36 hr.

Gentamicin was bactericidal to 19 (63%) out of the 29 strains tested. This action was rapid and resulted in a mean lowering of the bacterial population by 3 logarithms after 4 hr. However, 10 strains were able to survive in a medium containing 5 µg of gentamicin per ml for 36 hr, whereas vancomycin, though killing the bacteria more slowly, killed 26 (89%) of the strains tested (Fig. 2.) Two of the strains not killed by vancomycin were also resistant to gentamicin.

Kanamycin associated either with methicillin or cephalothin was shown by Bulger (2) to be a

suitable bactericidal regime for oxacillin-resistant staphylococci; cephalothin plus kanamycin was bactericidal for seven (77%) of nine strains. In the present study, this combination killed 22 (76%) of the 29 strains studied. Bulger's results therefore are confirmed here. Moreover, striking synergistic action has been observed for the 22 strains that were inhibited by cephalothin plus kanamycin, since none of them could be killed by cephalothin or kanamycin alone.

We investigated further the bactericidal activity of the combination cephalothin and gentamicin. Only one (3%) strain was not killed by this combination. The bactericidal action of cephalothin combined with gentamicin was extremely rapid, all the 28 sensitive strains being killed after 18 hr (Fig. 3). Since all these strains were resistant to cephalothin and 10 were resist-

ant to gentamicin, a striking synergistic action between the two antibiotics has been demonstrated for 9 strains among the 10 that were gentamicin-resistant. The strains of *S. aureus* that were not

killed by gentamicin were also tested for their susceptibility to a combination of oxacillin and gentamicin. Eight of the 10 strains were killed by the combination, demonstrating a distinct

FIG. 1. Standard killing curves (mean) obtained with oxacillin, cephalothin, and kanamycin for 29 strains of *Staphylococcus aureus*.

synergistic action between oxacillin and gentamicin against some strains of oxacillin-resistant *S. aureus*. No cross-resistance between oxacillin/gentamicin and cephalothin/gentamicin has been found here. The three strains of *S. aureus*

that were resistant to vancomycin were killed by either cephalothin/gentamicin or oxacillin/gentamicin.

Our study is in accordance with the results presented by other workers (1) who have shown

FIG. 2. Standard killing curves (mean) obtained with gentamicin and vancomycin for 29 strains of *Staphylococcus aureus*.

that vancomycin might be the best single drug available for the treatment of infections caused by oxacillin-resistant staphylococci. The use of vancomycin may be associated with serious un-

toward effects (5), and this drug is not readily available in many countries where the isolation of oxacillin-resistant staphylococci is commonplace. Under these circumstances it is under-

FIG. 3. Standard killing curves (mean) obtained with the combinations cephalothin/kanamycin, cephalothin/gentamicin, and oxacillin/gentamicin. The latter combination has been tested against 10 gentamicin-resistant strains only.

standable that gentamicin, which might be effective for the treatment of staphylococcal infections that are not oxacillin-resistant (7), should be also considered when one is dealing with oxacillin-resistant staphylococci.

Against strains that could not be killed by gentamicin alone, combinations of the latter drug with oxacillin or cephalothin were frequently synergistic, as defined by Jawetz (4), and resulted in rapid killing. No instances of antagonism were observed, but it should be emphasized that occasionally the inhibition of a penicillin by an aminoglycoside antibiotic has been reported.

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