APPLIED MICROBIOLOGY, March, 1965
Vol. 13, No. 2

Comparative In Vitro Activities of Lysostaphin and Other Antistaphylococcal Antibiotics on Clinical Isolates of *Staphylococcus aureus*

EDWARD F. HARRISON AND C. BRUCE CROPP

Chemotherapy Group, Department of Microbiology and Natural Products Research, Mead Johnson Research Center, Evansville, Indiana

Received for publication 7 October 1964

ABSTRACT

HARRISON, EDWARD F. (Mead Johnson Research Center, Evansville, Ind.), AND C. BRUCE CROPP. Comparative in vitro activities of lysostaphin and other antistaphylococcal antibiotics on clinical isolates of *Staphylococcus aureus*. Appl. Microbiol. **13**:212–215. 1965.—The in vitro activity of lysostaphin against clinical isolates of *Staphylococcus aureus* was determined by conventional tube-dilution methods. For comparison, minimal inhibitory concentration (MIC) values were also determined for penicillin G, ampicillin, methicillin, ristocetin, vancomycin, and erythromycin. Phage type and penicillinase and coagulase production were determined for each isolate. The MIC values for lysostaphin ranged from <0.047 to 12.5 μg/ml; 96% of the penicillinase-positive strains were inhibited by 1.56 μg/ml of lysostaphin, whereas 3.12 μg/ml of vancomycin and methicillin were required to attain the same degree of inhibition.

The unique bacterial enzyme, lysostaphin, discovered by Schindler and Schuhardt (1964) has a high degree of specificity for lysing staphylococci. These investigators found that lysostaphin lysed all staphylococcal cultures tested, including 54 strains of *Staphylococcus aureus* and 5 cultures of *S. epidermidis*. Cropp and Harrison (1964) tested 252 additional isolates, all of clinical origin, and found them highly susceptible to the lytic effect of lysostaphin. The strains tested were representative of three major *S. aureus* bacteriophage groups, including the phage-insensitive group, and varied in sensitivity to several clinically useful antibiotics. The present investigation was designed to evaluate quantitatively the antistaphylococcal activity of lysostaphin in comparison with other antibiotics used to treat infections caused by *S. aureus*.

MATERIALS AND METHODS

Clinical isolates. The 50 clinical isolates of *S. aureus* used in this study were obtained through the courtesy of William R. Cole, Department of Surgery, Wohl Hospital, St. Louis, Mo.

Phage typing. Phage typing was performed by Vera Gray at Wohl Hospital, by use of the standard phages recommended by the International Committee for Bacteriophage Typing of Staphylococci as reported by Blair and Carr (1960).

Minimal inhibitory concentration (MIC) test. The antistaphylococcal MIC for each antibiotic was determined by conventional tube-dilution methods, with Trypticase Soy broth (BBL). Each tube was inoculated with a diluted (10^5 organisms per milliliter) 24-hr broth culture of the isolate. Tubes were incubated at 37 C, and the MIC end point was determined after 18 hr according to the criterion of English and Gelwicks (1951).

Penicillinase. Penicillinase production for each isolate was determined by Haight and Finland's (1952) modification of the Gots method.

Coagulase test. Coagulase production was determined by the method of Fisk (1940) with fresh citrated rabbit plasma. Tubes were incubated at 37 C and read after 6 hr.

Antibiotics. The antibiotics used were: penicillin G (Nutritional Biochemicals Corp., Cleveland, Ohio), methicillin (Bristol Laboratories, Inc., Syracuse, N.Y.), ristocetin (Abbott Laboratories, North Chicago, Ill.), vancomycin HCl (Eli Lilly & Co., Indianapolis, Ind.), erythromycin glucoheptonate (Eli Lilly & Co.), ampicillin (Beecham, Betchworth, Surrey, England), and lysostaphin (Mead Johnson Research Center).

RESULTS AND DISCUSSION

The phage types and groups of the isolates were typical of those expected in a hospital environment (Table 1). Almost half (44%) of the isolates were susceptible to either phage types 80, 81, or a combination of the two.

All the isolates included in this study were coagulase-positive.

Staphylococcal penicillinase was not produced

by 18% of the cultures. This finding is confirmed by the low MIC values for penicillin G and ampicillin obtained with these specific isolates (Table 1). Higher MIC values were obtained with these two penicillins with all penicillinase-producing strains.

The destructive effect of penicillinase produced by the isolates on penicillin G and ampicil-

TABLE 1. *In vitro characteristics and antibiotic sensitivity of staphylococcal clinical isolates*

Isolate no.	Phage type	Phage group	Penicillinase production	Minimal inhibitory concn (μg/ml)			
				Penicillin G	Ampicillin	Erythromycin	Lysostaphin
4706	52/52A/80/81	I	+	>200	>200	0.39	0.39
4707	52/52A/80/81	I	+	>200	>200	0.39	0.39
4708	52/52A/80/81	I	+	>200	>200	0.39	0.39
4735	52/52A/80/81	I	+	>200	>200	>50	0.39
4758	52/52A/80/81	I	+	>200	>200	>50	0.78
4759	52/52A/80/81	I	+	>200	>200	>50	1.56
4690	52/52A/79/80	I	+	200	100	0.39	0.19
4694	29/52/52A/80	I	−	<0.39	<0.39	0.78	1.56
4695	52/52A/80	I	+	>200	>200	>50	0.19
4767	52/52A/80	I	−	1.56	1.56	0.39	0.39
4691	52A/80/81	I	+	>200	100	0.39	0.78
4696	52A/80/81	I	+	>200	>200	0.39	0.19
4705	52A/80/81	I	+	>200	>200	0.39	1.56
4721	80/81	I	+	>200	200	50	0.39
4726	80/81	I	+	>200	>200	>50	1.56
4731	80/81	I	+	>200	>200	0.39	0.39
4749	80/81	I	+	>200	>200	0.78	1.56
4751	80/81	I	+	>200	>200	0.39	1.56
4754	80/81	I	+	>200	>200	0.39	0.78
4757	80/81	I	+	>200	>200	>50	0.78
4762	80/81	I	+	>200	>200	0.39	0.78
4769	79/18	I	−	<0.39	<0.39	0.39	0.19
4764	18	I	+	200	200	0.39	0.095
4686	3B/3C	II	−	0.39	0.39	0.39	0.095
4709	3B/3C	II	−	<0.39	<0.39	0.39	0.19
4730	3A/71	II	+	12.5	6.25	0.78	0.095
4737	3B/3C/55/71/81	II	+	100	50	0.78	0.095
4750	55/71	II	−	<0.39	<0.39	0.78	0.19
4725	53	III	+	>200	>200	0.78	0.39
4713	54	III	+	>200	>200	0.19	<0.047
4733	54	III	+	200	100	0.39	0.19
4716	53/54	III	+	>200	200	6.25	0.39
4718	53/54	III	+	200	>200	12.5	0.39
4720	53/54	III	+	50	6.25	0.78	<0.19
4724	53/54	III	+	50	25	0.39	0.19
4727	53/54	III	+	>200	>200	>50	0.19
4728	53/54/83A	III	+	12.5	12.5	0.78	0.19
4752	53/54/83A	III	+	200	200	>50	0.39
4717	53/54/83A/83B	III	−	<0.39	<0.39	0.39	0.19
4719	53/83A/83B	III	−	<0.39	<0.39	0.78	0.19
4723	53/83A/83B	III	+	200	200	0.39	0.19
4755	53/83A/83B	III	+	200	200	0.78	0.19
4743	53/54/83A/83B	III	+	100	100	>50	12.5*
4736	53/54/83A/83B	III	+	>200	>200	>50	0.047
4753	75/53/54/83A	III	+	200	200	>50	0.095
4693	53/29/83A/54	III	−	<0.39	<0.39	0.39	0.095
4740	7/53/83A/83B	III	−	<0.39	<0.39	0.78	0.19
4770	83A/83B	III	+	50	12.5	0.78	0.78
4738	77/83A	III	−	<0.39	<0.39	0.78	6.25*
4741	53/54/83A	III	+	200	200	>50	0.095

* Single-colony isolates from 4743 had MIC values ranging from 0.047 to 12.5 μg/ml; 4738 subcultures had MIC values ranging from 0.78 to 12.5 μg/ml.

lin is demonstrated by the increased MIC values shown in Fig. 1. Methicillin, a penicillinase-resistant penicillin, had almost the same MIC values for both groups of isolates. The MIC values for penicillin G and ampicillin ranged from <0.39 to 1.56 μg/ml for penicillinase-negative cultures, representing 18% of the isolates, and 12.5 to >200 μg/ml for the remaining 82% of the penicillinase-positive strains. The antistaphylococcal activity of methicillin ranged from 1.56 to 6.25 μg/ml for both groups of isolates. These values agree with the findings of Klein and Finland (1963), Brown and Acred (1960), and White and Varga (1961).

Vancomycin and ristocetin were consistent in their antistaphylococcal activities and varied only over a narrow range of concentrations. All strains were sensitive to 1.56 to 3.12 μg/ml of vancomycin and 6.25 to 12.5 μg/ml of ristocetin. Reedy and Shaffer (1957) reported that the majority of the *S. aureus* strains tested were sensitive to 3.12 μg/ml of vancomycin; our strains were likewise sensitive to 3.12 μg/ml (86%). The MIC for ristocetin was 12.5 μg/ml for the majority of the isolates (90%), in agreement with the values reported by Grundy et al. (1957).

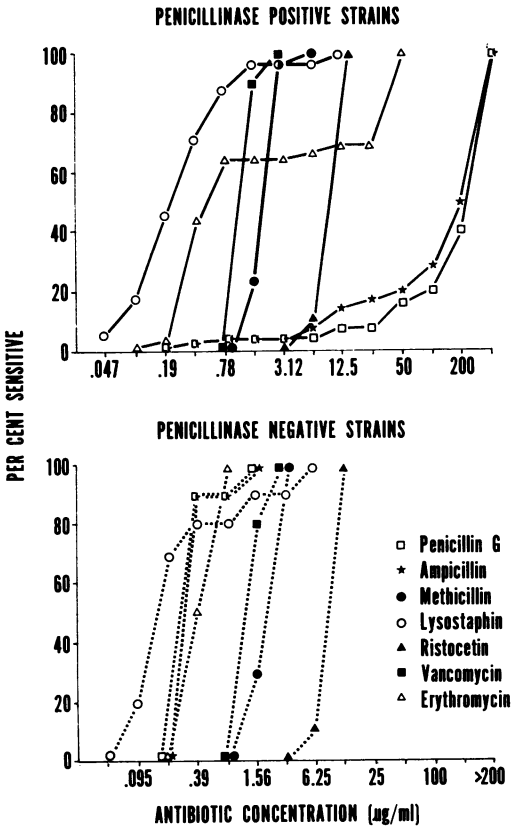

FIG. 1. *Comparison of staphylococcal susceptibility to lysostaphin and other antistaphylococcal antibiotics.*

The sensitivity of the isolates to erythromycin was divided into two ranges, the very sensitive group (0.19 to 0.78 μg/ml, 70%) and the resistant group (6.25 to 50 μg/ml, 30%). This same pattern of increased resistance to erythromycin was shown by Wise et al. (1955). The most resistant strains were also found to be penicillinase producers.

The MIC values for lysostaphin, on the other hand, ranged from <0.047 to 12.5 μg/ml. At a MIC of 1.56 μg/ml, 96% of the penicillinase-positive strains were inhibited by lysostaphin. The penicillinase-negative strains were likewise sensitive to lower concentrations of lysostaphin than of the other antistaphylococcal antibiotics. One isolate (4743) required 12.5 μg/ml of lysostaphin to inhibit its growth, and another isolate (4738), 6.25 μg/ml. In view of the apparent relative lack of sensitivity of these two specific isolates to lysostaphin, the homogeneity of these cultures was determined by studying single-colony isolates. Both clinical isolates were lyophilized shortly after being received in the laboratory and were held at 5 C prior to being reconstituted. Both cultures were streaked on Staphylococcus Medium 110 (Difco), and six single colonies were picked from each isolate. The lysostaphin MIC values for the 4743 subcultures ranged from 0.047 to 12.5 μg/ml (0.047 μg/ml for one; 0.09 μg/ml, two; 0.78 μg/ml, two; and 12.5 μg/ml, one). For the 4738 subcultures, MIC values ranged from 0.78 to 12.5 μg/ml (0.78 μg/ml for one; 1.56 μg/ml, one; 6.25 μg/ml, one; and 12.5 μg/ml, three). All subcultures of both isolates fermented sucrose, dextrose, and mannitol in addition to being *S. aureus* phage-typable. When lysostaphin was added to a viable-cell suspension of either the original isolates (4738, 4734) or their subcultures, the cells were rapidly lysed. These isolates fall into the group III classification, which is the phage group that is most commonly associated with the emergence of antibiotic-resistant strains of staphylococci according to Jevons (1961). The elevated MIC values found with these two isolates are not believed to be significant, as similar observations are reported by Knox (1961) for methicillin without any serious clinical problems arising from resistant strains. Kjellander, Klein, and Finland (1963) made a similar observation on the effects of inoculum size on the MIC values of methicillin for methicillin-resistant strains of *S. albus*. They postulated that the elevated MIC

values were due to the presence of a small number of cells with inherent methicillin resistance.

These studies show that lysostaphin is a potent antistaphylococcal antibiotic. Because of its unique property of lysing actively growing, dormant, or killed staphylococci equally well, in contrast to most clinically useful antibiotics whose mode of action is restricted to actively metabolizing cells, lysostaphin should be a significant addition to the chemotherapeutic agents useful for the treatment of staphylococcal infections.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of M. E. Fuquay and R. L. Stratman.

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