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Mathematical Modeling of Antimicrobial Susceptibility Data of *Staphylococcus haemolyticus* for 11 Antimicrobial Agents, Including Three Experimental Glycopeptides and an Experimental Lipoglycopeptide

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Antimicrobial MIC data were obtained for 96 strains of *Staphylococcus haemolyticus* and the following 11 antimicrobial agents: methicillin, gentamicin, rifampin, fusidic acid, ciprofloxacin, vancomycin, teicoplanin; three experimental glycopeptides, MDL 62,873, MDL 62,208, and MDL 62,224; and an experimental lipoglycopeptide, ramoplanin. Resistance to methicillin and gentamicin was present in over 50% of the strains, although resistance to the other agents was present in less than 10%. It is shown how application of mathematical modeling techniques can add to the understanding of such MIC data. MICs of methicillin and gentamicin were highly correlated, suggesting that evolutionary pressures for development of resistance to these agents were similar. The structural relationships among the glycopeptides were accurately reflected in their spatial relationships within the model. MICs of ramoplanin were negatively correlated with MICs of some other antimicrobial agents, particularly gentamicin, suggesting that this agent is more active against gentamicin-resistant strains. Methicillin-resistant strains were more tightly clustered than were methicillin-susceptible strains, suggesting that methicillin-resistant strains were more closely related to each other than were methicillin-susceptible strains. Mathematical modeling techniques enable more detailed analysis of MIC data.

Staphylococcus haemolyticus is one of the more commonly isolated coagulase-negative *Staphylococcus* species in hospital practice (4, 6, 15). Indeed, it is the second most common coagulase-negative *Staphylococcus* species received at the Division of Hospital Infection, London, England, forming 15% of all such strains. Its potential significance as a nosocomial pathogen is further enhanced by the finding that many strains are resistant to multiple antimicrobial agents (6–8, 15). Furthermore, reduced susceptibility to vancomycin in *S. haemolyticus* has also been described (17). In this report, we describe the results of a study of the antimicrobial susceptibility of *S. haemolyticus* to 11 established and experimental compounds.

Most studies of in vitro antimicrobial susceptibility data are analyzed by fairly standard statistical methods. In particular, data are frequently presented in the form of the range of MICs in the population studied, usually with the MICs for 50 and 90% of the isolates tested (MIC_{50} and MIC_{90}). Other methods for presentation of data may include the cumulative percentage susceptible to increasing concentrations of an antimicrobial agent. These descriptive statistical methods merely demonstrate how effective each agent is against the microbial population under investigation. While this is satisfactory in many cases, a considerable amount of information is lost by such cursory analysis. This report suggests a more searching statistical approach to the analysis of such MIC studies. It is further shown how more detailed statistical analysis can give additional information concerning the population under investigation and the relationships among the antimicrobial agents themselves.

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MATERIALS AND METHODS

Strains. Ninety-six strains of *S. haemolyticus* were included in the study. All strains had been isolated from within the United Kingdom and had been sent to the Division of Hospital Infection for identification and typing. Strains that may have been closely related to other strains already included in the set of data because they had been isolated from the same patient or from different patients on the same ward at the same time were excluded from the study. Of these 96 strains, 53 were from blood cultures, 20 were from cerebrospinal fluid samples, 9 were from intravascular devices, 11 were from wound swabs, and 3 were from urine. Identification was by biochemical methods described previously (13), and classification into biotypes was as described by Marples et al. (14).

Susceptibility testing. The following 11 antimicrobial agents were examined in this study: methicillin, gentamicin, rifampin, fusidic acid, ciprofloxacin, vancomycin, teicoplanin; three experimental glycopeptides, MDL 62,873 (syn CTA-A-1), MDL 62,208 (syn TD-A-3), and MDL 62,224 (syn TD-A-4); and an experimental lipoglycopeptide, ramoplanin (syn A16686). The experimental compounds were provided by Merrell Dow Research Institute, Lepetit Research Center, Gerenzano, Italy. MIC testing was done by an agar dilution method with multipoint inoculation onto the agar plates, each inoculum consisting of 5×10^5 CFU (19). All plates were incubated overnight at 37°C, except for methicillin plates, which were incubated at 30°C. Strains were classified as resistant to a given antimicrobial agent by using the following breakpoints (micrograms per milliliter): methicillin, >8; gentamicin, >4; rifampin, >1; fusidic acid, >1; ciprofloxacin, >4; vancomycin, >4; teicoplanin, >4 (20).

TABLE 1. MIC_{50}s, MIC_{90}s, and MIC ranges of 11 antimicrobial agents for *S. haemolyticus*

Antimicrobial agent	MIC (mg/liter)		
	50%	90%	Range
Teicoplanin	2	4	≤0.12–16
Vancomycin	2	2	0.25–4
Ramoplanin	0.5	1	≤0.12–2
MDL 62,873	1	2	≤0.12–2
MDL 62,208	0.5	1	≤0.12–2
MDL 62,224	0.5	1	≤0.12–2
Methicillin	16	>64	≤0.5–>64
Gentamicin	8	>32	≤0.06–>32
Fusidic acid	≤0.06	1	≤0.06–8
Rifampin	≤0.06	≤0.06	≤0.06–2
Ciprofloxacin	0.25	0.5	≤0.06–1

Statistical methods. Before multivariate analysis, each MIC was transformed to its $\log_2$ value to approximate the results to the standard distribution; i.e., an MIC of 8 mg/liter was transformed to 3 and an MIC of 0.25 mg/liter became −2. The results for each antimicrobial agent were then standardized to give (i) zero mean by subtracting the mean MIC from all of the MICs of that antimicrobial agent and (ii) unitary standard deviation by dividing each value by the standard deviation.

Geometric modeling of the strain relationships was performed by the principal coordinate analysis (PCO) program described by Davies (3). The program listing given by Davies was transcribed onto an Atari ST microcomputer by using the Prospero Fortran compiler (Prospero Software, London, England). Geometric modeling of the relationships between the antimicrobial agents was performed after transposition of the standardized primary data matrix.

PCO is a statistical technique that is closely related to principal component analysis. Many statistical packages are capable of performing principal component analysis and PCO (2). The object of principal component analysis is to take p real variables and determine various combinations of these variables to produce indices that are uncorrelated (12). The first principal axis can be considered a line such that if all members of the population are projected onto that line, the greatest possible variation in the population is represented. The second principal axis is the line that shows the next greatest variation, provided that this axis is uncorrelated with the first, and so on. In PCO, the position of each population member of the first few principal axes can be used to plot two- and three-dimensional scatter plots. A scatter plot based on the first and second principal axes is the two-dimensional representation of the population that best shows the variation within the population, and similarly, a three-dimensional scatter plot of the first three principal axes is the best three-dimensional representation. This enables the relationships among entities to be summarized by display on two- or three-dimensional scatter plots for easy visual inspection, even though the entities themselves may have been defined by many characters. The axis units are not given on these scatter plots, as this would provide little extra information. The relative closeness of the entities to one another is of primary interest.

RESULTS

Standard analysis. The MIC_{50} and MIC_{90}, along with the range of MICs, are shown in Table 1. Table 2 shows the MIC_{50}, MIC_{90}, and MIC range for methicillin-susceptible and -resistant strains. On the basis of the breakpoints, 51% of the strains were resistant to methicillin, 53% were resistant to gentamicin, 8% were resistant to fusidic acid, 1% were resistant to rifampin, and 9% were resistant to teicoplanin. All strains were susceptible to ciprofloxacin, vancomycin, and the experimental compounds. Among the methicillin-resistant strains, 92% were resistant to gentamicin, 12% were resistant to fusidic acid, 2% were resistant to rifampin, and 12% were resistant to teicoplanin.

TABLE 2. MIC_{50}s, MIC_{90}s, and MIC ranges of 10 antimicrobial agents for methicillin-susceptible and -resistant strains of *S. haemolyticus*

Antimicrobial agent	MIC (mg/liter) for:					
	Methicillin-susceptible strains			Methicillin-resistant strains		
	50%	90%	Range	50%	90%	Range
Teicoplanin	1	4	≤0.12–16	2	4	≤0.12–8
Vancomycin	1	2	0.25–4	2	2	1–2
Ramoplanin	0.5	1	≤0.12–2	0.5	0.5	0.25–1
MDL 62,873	0.5	1	≤0.12–2	1	2	0.25–2
MDL 62,208	0.5	1	≤0.12–2	1	1	0.5–2
MDL 62,224	0.5	1	≤0.12–2	1	1	0.25–2
Gentamicin	0.25	0.5	≤0.06–>32	16	>32	≤0.06–>32
Fusidic acid	≤0.06	0.25	≤0.06–8	≤0.06	2	≤0.06–8
Rifampin	≤0.06	≤0.06	≤0.06–0.5	≤0.06	≤0.06	≤0.06–2
Ciprofloxacin	0.25	0.5	≤0.06–1	0.25	0.5	0.12–1

Relationships among antimicrobial agents. The geometric representation of the relationships among the antimicrobial agents is shown in the three-dimensional scatter plot shown in Fig. 1. The close relationship among the three experimental compounds (MDL 62,208, MDL 62,224, and MDL 62,873) and teicoplanin, from which they are derived, can be seen. It is notable that MDL 62,873 is closer to teicoplanin than to MDL 62,208 and MDL 62,224 and that the latter are situated particularly close together. By contrast, the experimental compound ramoplanin is situated remote from other agents. Surprisingly, the model also shows a relatively close relationship between methicillin and gentamicin, reflecting the finding that methicillin-resistant strains are also likely to be resistant to gentamicin (Table 2).

Further information regarding the relationships among the antimicrobial agents can be obtained from examination of the intermediate stages in PCO. The correlation half matrix (Table 3) confirmed the findings shown more graphically in Fig. 1. However, a point of interest is that the correlation coefficients between ramoplanin and several of the other antimicrobial agents, particularly gentamicin, were frequently negative. Thus, it appears that ramoplanin is more active against strains that are resistant to gentamicin.

Relationships among strains. Figure 2 shows the scatter plot of the first two principal coordinates of the relationships among strains. As might have been expected, methicillin-susceptible and -resistant strains tended to occupy different areas of the model. However, it is notable that methicillin-susceptible strains tended to occupy a rather more diffuse area than did resistant strains, which formed a relatively closely defined group. In other words, the scatter plot showed that methicillin-resistant strains formed a tighter cluster than did methicillin-susceptible strains.

DISCUSSION

The use of multivariate statistical methods, as described here, assisted in the analysis of these MIC data. Standard

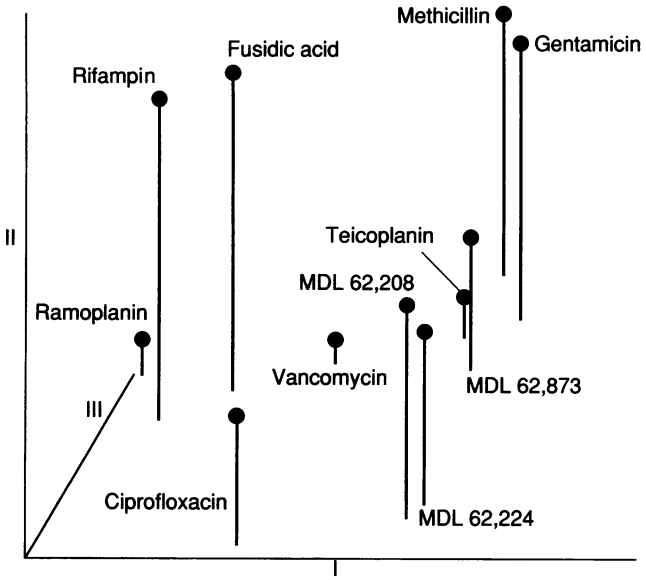

FIG. 1. Three-dimensional scatter plot of the relationships among 11 antimicrobial agents. The boundaries of the scatter plot are represented by the horizontal axis (I), the vertical axis (II), and the oblique axis (III). Axis III is shown as oblique; because of the effect of perspective, the axis should go straight into the page at right angles to the other two axes. These represent the first three principal axes. The plane defined by axes I and III could be considered the floor of a room, and the plane defined by axes II and III could be considered the left hand wall. The position of each antimicrobial agent within this hypothetical room is shown by the position of its representative dot. The position on axes I and III, or the place on the floor, is given by the base of the line below each dot, while the position on axis II, or height above the floor, is given by the length of each line. The interpretation of this model is discussed further in the text.

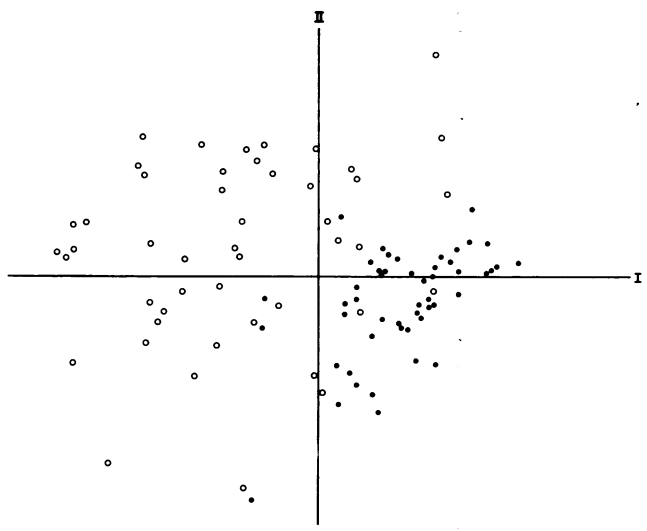

FIG. 2. Two-dimensional scatter plot of the relationships among methicillin-susceptible (○) and -resistant (●) strains of *S. haemolyticus*. Axes I and II represent the first two principal axes. The interpretation of this model is discussed in the text.

analysis, with the production of long tables of MIC results, although necessary, can hide important information about the strain populations and antimicrobial agents under investigation. However, it is important to remember the limitations of these geometric models. The results of PCO are critically dependent on the data and may not be quantitatively generalizable outside the set of data, although qualitatively they can usually be so generalized (2). Thus, the results obtained depend on the strains included in the study and on the antimicrobial agents used in their characterization. Care was taken to avoid bias due to strain selection by not excluding strains of *S. haemolyticus*, except when these may have been related to strains already included in the set of data. Furthermore, all of the antimicrobial agents in the study were potentially effective against strains of *S. haemolyticus*. Nevertheless, it must be remembered that the models obtained offer a qualitative rather than a quantitative interpretation of the data. Geometric modeling of multivariate data is an essentially descriptive tool; it cannot be used to prove a hypothesis, although it can be useful in the formation of hypotheses (2, 5, 10, 12, 16).

Such statistical techniques are frequently used in the biological sciences to assist in the description of the structure of a population and the phylogenetic relationships among individual members of that population (1, 16). Their use to describe relationships among characteristics used to examine populations is less common. In evolutionary genetics, there are several explanations for the finding of a strong correlation between phenotypic characteristics and their proximity in such models (9, 18). Two highly correlated characteristics may be different ways of measuring similar things, may be genetically linked, or may be subject to similar evolutionary pressures. The application of these principles to the study of MIC data would be that antimicrobial MICs would be highly correlated if the antimicrobial agents were essentially the same compound, with only minor structural differences; if resistance to them were genetically linked by, for example, joint carriage on a single plasmid; or

TABLE 3. Coefficients of correlation between antimicrobial agents

Antimicrobial agent	Coefficient of correlation with:									
	Vancomycin	Ramoplanin	MDL 62,873	MDL 62,208	MDL 62,224	Methicillin	Gentamicin	Fusidin	Rifampin	Ciprofloxacin
Teicoplanin	0.595	0.154	0.716	0.332	0.443	0.418	0.441	−0.428	−0.258	0.205
Vancomycin		0.345	0.520	0.175	0.215	0.152	0.168	−0.087	−0.025	0.203
Ramoplanin			0.061	−0.206	−0.149	−0.143	−0.359	−0.062	0.072	−0.024
MDL 62,873				0.622	0.588	0.532	0.596	0.005	0.025	0.207
MDL 62,208					0.854	0.296	0.384	−0.024	0.067	0.226
MDL 62,224						0.297	0.401	0.034	−0.146	0.240
Methicillin							0.694	0.127	0.022	−0.031
Gentamicin								0.130	0.014	0.087
Fusidin									0.190	−0.023
Rifampin										−0.085

if the evolutionary pressures for resistance to both antibiotics were similar. This last point probably explains the otherwise surprising degree of correlation between methicillin and gentamicin. Both antimicrobial agents are used a great deal in the United Kingdom, particularly in specialist units and often in combination. Thus, strains of *S. haemolyticus* that experience evolutionary pressure to develop methicillin resistance also experience evolutionary pressure to develop gentamicin resistance.

The model accurately predicted the structural relationships among the experimental compounds. The distant position of A-16686 in the model reflects its rather distinct structure (J. K. Kettering, R. Ciabalti, G. Winters, G. Tamborini, and B. Cavalleri, Proc. 2nd Int. Symp. New Bioactive Metab. Microorg., abstr. no. 103, 1988). Furthermore, the model accurately predicted the relationships of the experimental glycopeptides to teicoplanin. MDL 62,873, an amide derivative of the teicoplanin A2 complex, is more similar to teicoplanin than to MDL 62,208 and MDL 62,224, which are amide derivatives of the aglycon of the teicoplanin A2 complex (11). Also, MDL 62,873 is more similar to MDL 62,208, with which it shares an identical carboxamide group.

The use of this type of statistical modeling could have benefits in the development of new antimicrobial agents. The development of new compounds is often by modification of existing agents in the hope that the derivatives will exhibit improved antimicrobial activity or pharmacokinetics. The application of these modeling techniques will provide a rapid indication of whether a structural alteration is likely to have any significant effect on the antimicrobial activity of the new agent compared with its parent drug.

One interesting finding was the negative correlation between ramoplanin and several of the other agents, particularly gentamicin. Ramoplanin is a lipoglycopeptide which acts on cell walls and is likely to be used only for topical therapy. These results suggest that cell walls of gentamicin-resistant strains may be altered in some way such that they are more susceptible to ramoplanin. Although it could be argued that this suggestion may result from reading too much into a simple correlation, the modeling process has certainly pointed to a hypothesis that would be well worth investigating. If this hypothesis proves to be true, then the widespread use of ramoplanin will be unlikely to contribute to an increased prevalence of resistance to systemic antimicrobial agents.

A further interesting observation was that methicillin-resistant strains tended to occupy a more compact area of the model than did methicillin-susceptible strains. One explanation for this could be that most of the methicillin-resistant strains of *S. haemolyticus* are phylogenetically closely related and are derived from a single parent cell in the relatively recent past. The geometric models used in this study have raised hypotheses which merit further investigation. Some further evidence for this hypothesis comes from the finding that 45 (92%) of the methicillin-resistant strains are of a single biotype (SVI [1]), compared with 22 (47%) of the methicillin-susceptible strains.

We have confirmed previous findings that *S. haemolyticus* is frequently resistant to methicillin and gentamicin. This finding has added significance in that most of the strains included in this study were isolated from deep sites, such as blood or cerebrospinal fluid, where their pathogenic potential would have been higher. Nevertheless, strains of *S. haemolyticus* from potentially serious infections remain susceptible to the glycopeptides and ciprofloxacin.

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