ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1975, p. 614-621

Vol. 7, No. 5
Printed in U.S.A.

Epidemiology of Antibiotic and Heavy Metal Resistance in Bacteria: Resistance Patterns in Staphylococci Isolated from Populations Not Known to be Exposed to Heavy Metals

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Received for publication 26 November 1974

Staphylococci were isolated from clinical specimens obtained from patients not known to be exposed to abnormal levels of heavy metals. The antibiotic and heavy metal resistance patterns of these strains were determined by using a disk diffusion test and computer sorting. Though not absolute, an association of resistance to mercury and tetracycline in coagulase-negative strains was found, in contrast to resistance to copper and penicillin in coagulase-producing strains. A high degree of correlation was observed between the resistance to phenyl mercury and inorganic mercury, but no correlation was obtained between resistance to methylmercury and other metals. In general, strains resistant to many agents were usually coagulase negative. A possible mechanism and implications of these associations are considered.

The patterns of resistance to antibiotics and heavy metals have intrigued investigators since Moore (22) made the observation that one could divide staphylococci into pathogenic and nonpathogenic classes on the basis of resistance or susceptibility to a critical concentration of mercuric ions. Correlations have subsequently been established between resistance to mercuric ions and many of those characteristics implicated in the pathogenicity of these organisms. Hospital populations of staphylococci have been shown to have a large fraction of mercury-resistant strains (25), although no correlation could be demonstrated between resistance to mercuric ion and production of extracellular enzymes (9), or characteristics likely to promote survival in the hospital environment (13). Mercury resistance has been associated, however, with resistance to penicillin and tetracycline (10, 22), resistance to multiple antibiotics, to phage type (10), to pigment production (30), to high levels of penicillinase production (27), and to production of penicillinase type A (7).

Although the problem of the increased frequency of mercury resistance in pathogenic strains of staphylococci is not yet resolved, the association of mercury resistance with various other resistance determinants has been partially explained by the elegant description of the penicillinase plasmid and its associated resistance determinants by Novick (23) and his collaborators. Acting on the observation of Barber (4) that penicillinase production could be lost by penicillin-resistant strains on storage, Novick described (23) the genetic determinants of penicillinase as being carried on a plasmid which can also carry resistance to several inorganic salts, including cadmium (24) and in some cases resistance to macrolide antibiotics (21).

In staphylococci, genes for antibiotic and heavy metal resistance have been located both on plasmids and on the chromosome. For example, plasmid-controlled antibiotic resistance has been reported for tetracycline, chloramphenicol, and kanamycin (23), as well as for lincomycin (1), fusidic acid (17), and methicillin (6). Other determinants carried by plasmids in *Staphylococcus aureus* include bacteriocins linked to metal resistance (14). In coagulase-negative, mannitol-positive *Staphylococcus epidermidis*, oxacillin and kanamycin resistance have been simultaneously eliminated at altered pH levels. In the same strain, the penicillinase plasmid carried determinants for mannitol and β-glucoside uptake, ribose fermentation, and phospho-β-glucosidase activity (28). In another study of *S. epidermidis* (3), the determinants for inorganic ion resistance were shown to be unlinked to penicillinase production, either in natural patterns or by elimination. Although the clinically significant forms of resistance to antibiotics are usually plasmid-borne, the determinants for penicillinase (2, 26) and streptomycin (16) can be either on the chromosome or a plasmid. Thus, the location of these traits is variable and strain dependent.

All of these linkage studies are subject to

well-known experimental limitations (23). Only recently has conclusive evidence for the plasmid nature of the penicillinase gene, in some strains, been presented (19) by purifying covalently closed circular deoxyribonucleic acid from penicillinase-producing strains and by introducing this material to recipient strains via transformation, with subsequent demonstration of covalently closed circular deoxyribonucleic acid in the transformed penicillinase-producing recipients. This technique combined with the development of the chromosomal genetics of staphylococci should allow the unequivocal assignment of a given marker to chromosomal or plasmid classes.

Although inherent problems render the interpretation of the resistance patterns in staphylococcal strains difficult, the mechanisms by which antibiotic resistance patterns arise is obviously of such importance as to require further effort. Prior to studies on the molecular biology and biochemistry of heavy metal resistance in individual strains, as well as the effects of heavy metal loads on microorganisms inhabiting man, it was considered essential to determine the patterns of antibiotic resistance and heavy metal resistance of microorganisms inhabiting humans not known to have large amounts of heavy metals in their bodies. This publication describes the computer-sorted patterns of antibiotic and heavy metal resistance in such a population of staphylococcal strains from Strong Memorial Hospital, Rochester, N. Y.

MATERIALS AND METHODS

Bacterial strains. Staphylococcal strains were isolated from exudate swabs submitted to the Diagnostic Microbiology Laboratory of Strong Memorial Hospital, Rochester, N. Y. Each sample was obtained from a different patient.

Determination of coagulase production and mannitol fermentation. Free coagulase was determined by inoculating 0.5 ml of calf serum in a shell vial with the strain to be tested, then incubating for 3 h at 37 C and observing coagulation. Tubes which were not coagulated at 3 h were reincubated for 14 h for further observation. Mannitol fermentation was observed by transferring overnight Penassay broth (Antibiotic medium no. 3, Difco) cultures to grids on mannitol salt agar (BBL), incubating for 36 h at 37 C, and recording bright yellow zones as positive.

Susceptibility testing for antibiotics. Susceptibility testing for antibiotics was performed by the disk diffusion (8) technique. Isolated colonies were diluted in saline to yield a nearly confluent lawn when swabbed onto 150-mm plates containing a layer of Mueller-Hinton agar with 5% sheep blood. Sensitivity disks (Sensi-discs, BBL) containing penicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), tetracycline (30 μg), ampicillin (10 μg), cephalothin (30 μg), lincomycin (2 μg), and methicillin (5 μg) were placed on the agar and the cultures were incubated overnight at 37 C. The susceptibility or resistance to each antibiotic was determined from a measurement of the zone of inhibition of growth.

Susceptibility to heavy metals. Susceptibility to heavy metals was tested by a modification of the disk diffusion technique described above for antibiotics. For mercuric chloride, phenyl mercuric chloride, cupric chloride, and cadmium chloride, 20 μl of an appropriate solution was added to sterile, blank, paper sensitivity disks (BBL) and dried for 2 to 3 h at 37 C. Each disk contained an appropriate amount (Table 1) of agent to give a zone of inhibition. The size of the zone was used to differentiate between resistant and susceptible strains. The strains to be tested were grown overnight at 37 C in 5 ml of Penassay broth and inoculated to Mueller-Hinton agar plates as described for antibiotic susceptibility testing, and the dried, metal salt-containing disks were placed on the inoculated plates. An untreated disk was also placed on the plate and 10 μl of a methyl mercuric chloride solution was added immediately. The plates were inverted and incubated at 37 C for 24 h. After incubation, the sizes of the zones of inhibition of growth were measured, using a clear plastic gauge, and the diameters of the zone were recorded on computer coding forms, along with antibiotic resistance patterns and source identifier data. The coded data were converted to punched-card format then stored and manipulated by an ad hoc computer program written in Fortran IV, as described elsewhere (11). Briefly, the punched card data were transferred to a magnetic tape and the distribution of zone diameters versus their frequency for each metal was plotted by the program. This allowed the assignment of a critical diameter to separate the resistant and susceptible populations for each metal. The program used this critical value to assign metal resistance and susceptibility patterns for each strain tested. These patterns could be compared with the antibiotic patterns for analysis of association between the various resistance determinants, as well as other characteristics of the strains.

RESULTS

Determination of populations resistant and susceptible to various metals. To define resistant and susceptible strains for each heavy metal tested, frequency distribution plots of the diameters of the zones of inhibition were examined as described above. For each of the metals, with the exception of methylmercury, a bimodal distribution allowed the separation of resistant and susceptible populations. In addition, each strain resistant to phenyl mercury was found to also be resistant to mercuric ions and vice versa.

Correlation of resistance to different metallic ions. Diameters of the zones of inhibition were compared in a regression analysis for each pair of metal cations to determine if there was any relationship between the sizes of zones for one metal compared with those of the other metal compounds (11). The correlation coeffi-

cient (r) and the normal deviate (z) of this correlation coefficient were calculated for each of the pairs of metals (Table 2). The values for r range from 0.81 for the Hg^{2+} ion/phenyl-Hg^{+} pair to a low of 0.26 for the comparison of Cu^{2+} ion and phenyl Hg^{2+} ion. A high degree of correlation indicates that a strain which can resist one of the metals can also resist the other. Furthermore, a strain which cannot resist the first metal also cannot resist the second metal and thus larger zones of inhibition are produced. For example, there is a high degree of correlation between resistance to phenyl mercuric ion and mercuric ion and a low degree of correlation of resistance to either of these two metals with resistance to Cu^{2+} ions. In pairs of metals with a very high correlation, only one set of data need be gathered and maintenance of the high degree of correlation from trial to trial serves as a valuable control. The overall pattern of correlations also serves as an internal control for the entire disk diffusion test system.

The correlation between zone diameters for methyl mercury and other metals did not vary significantly. This might well have been a function of the absence of populations definable as resistant or susceptible to methyl mercury, with no resistance character to correlate with other metal resistance characters.

Correlation of resistance to antibiotics and metal ions. Because every strain resistant to phenyl mercury was also resistant to mercuric ions and because no strains resistant to methyl mercury were found, further analysis of heavy metal resistance patterns involved only resistance to Hg^{2+}, to Cu^{2+}, and to Cd^{2+} ions. The staphylococcal strains were tested for their resistance or susceptibility to seven antibiotics and these three heavy metals (Table 3). In this analysis, each of the agents was considered independently of the others in its class. Therefore, strains resistant to Hg^{2+} might also be resistant to Cd^{2+} or Cu^{2+}, and strains resistant to penicillin might also be resistant to any of the other antibiotic agents. As a result the sum of each column and row is larger than the figure given as total for that agent. Of a total population of 704, 226 are resistant to at least Hg^{2+} ions, and 488 are resistant to Cu^{2+}, whereas 260 are resistant to Cd^{2+}. Nearly equal numbers are susceptible to all metals (112) as are susceptible to all antibiotics (133), but these are not the same fraction, as only 36 are susceptible to all of the agents tested. The largest class contains the 539 strains resistant to penicillin and other major classes are those resistant to at least erythromycin or tetracycline with 92 out of 704 and 170 out of 704, respectively. Therefore, it would appear productive to examine more closely the relationships between penicillin, erythromycin, and tetracycline resistance as well as associations with the various metal resistance patterns. In the populations resistant to at least penicillin, there is a ratio of Hg^{2+}-resistant to Cu^{2+}-resistant strains of 179 to 386 or 0.464. For tetracycline-resistant strains, the ratio is 118 to 106 or 1.11. It would appear that strains resistant to tetracycline have an increased probability of being resistant to mercury; note a similar association exists between penicillin resistance and copper ion resistance. A different analysis demonstrates that the association of copper ion and penicillin resistance might well be an artifact generated by the large fractions of the population which are resistant to these two agents. The fraction of each metal-resistant class in the populations resistant to penicillin, to erythromycin, or to tetracycline is calculated in Table 4. For example, of the 539 strains resistant to at least penicillin, 0.33 are resistant to Hg^{2+}, 0.72 are resistant to Cu^{2+}, and 0.37 are resistant to Cd^{2+}. For Hg^{2+} ions, the fraction of each antibiotic-resistant population varies greatly, from 0.33 for penicillin to 0.825 for erythromycin and 0.695 for tetracycline; however, the fraction for each antibiotic population remains fairly constant for Cu^{2+} ions and Cd^{2+} ions. Thus, the association between tetra-

TABLE 1. *Amount of metal required for assay*

Metal	μmol/disk
Cu^{2+}	20.0
Cd^{2+}	2.0
Hg^{2+}	0.038
Phenyl mercury	0.008
Methyl mercury	0.0004

TABLE 2. *Correlation of metal resistances*

Test metal	Constant	Analysis with:			
		Phenyl-Hg^{2+}	Methyl-Hg^{2+}	Cu^{2+}	Cd^{2+}
Hg^{2+}	r[a]	0.81	0.48	0.31	0.45
	z[b]	1.14	0.52	0.32	0.49
Phenyl-Hg^{+}	r		0.53	0.26	0.38
	z		0.59	0.27	0.40
Methyl-Hg^{+}	r			0.44	0.43
	z			0.47	0.46
Cu^{2+}	r				0.54
	z				0.56

[a] r, Correlation coefficient for the zone sizes for each pair of metals.

[b] z, Normal deviate of r. $z = 0.5(\log_e(1 + r) - \log_e(1 - r))$.

TABLE 3. *Correlation of resistance traits for antibiotics and metal ions*

Antibiotic resistance	Metal resistance[a]				
	Total	None	Hg^{2+}	Cu^{2+}	Cd^{2+}
None	133	36	29	81	46
Penicillin	539	71	179	386	201
Chloramphenicol	17	1	15	8	9
Erythromycin	92	5	76	72	50
Tetracycline	170	21	118	106	78
Cephalothin	6	2	2	4	0
Lincomycin	36	4	29	27	14
Methicillin	48	5	35	30	15

[a] Of the 704 strains tested, 112 were susceptible to all three metals, 226 were resistant to Hg^{2+}, 488 were resistant to Cu^{2+}, and 260 were resistant to Cd^{2+}. In this analysis, each of the metals was considered independently. Therefore the sum is greater than 704 because many of the strains were resistant to more than one metal.

TABLE 4. *Calculated fractions of each metal resistance class for each of the populations resistant to at least penicillin, erythromycin, or tetracycline*

Antibiotic	Fraction[a]			
	Total population	Hg^{2+}	Cu^{2+}	Cd^{2+}
All classes	704	0.32	0.69	0.37
Penicillin	539	0.33	0.72	0.37
Erythromycin	92	0.83	0.78	0.54
Tetracycline	170	0.69	0.62	0.46

[a] Fraction of strains in each antibiotic resistance class resistant to each metal.

cycline and mercury resistance is confirmed, whereas for resistance to copper and probably cadmium, the associations, if any, are more difficult to demonstrate.

Correlation of metal-resistance patterns with single antibiotic resistance traits. To further examine these relationships, the classes resistant to none or to only one of the antibiotics were subdivided into each of the eight possible resistance classes for Hg^{2+}, Cu^{2+}, and Cd^{2+}. Significant numbers were only found for those classes resistant to no antibiotics, to penicillin alone, and to tetracycline alone (Table 5). Note the very large number (353 out of 704) that are resistant only to penicillin as compared to the relative scarcity (18 out of 704) of strains resistant to tetracycline alone. By using a 2×8 contingency table to calculate X^2, it was found that significant differences existed in the distributions of metal resistance patterns for the three classes of antibiotic resistance. The differences are partially explained by the decrease in fractions susceptible to all metals (36 out of 133 in totally susceptible strains) when the strain carries penicillin resistance (48 out of 353) or tetracycline resistance (1 out of 18). In addition, there was an increase in the fraction of strains resistant only to copper (35 out of 133 in strains susceptible to all antibiotics) when penicillin resistance is present (153 out of 353). With the introduction of tetracycline resistance in the population, there is an increase in the fraction of strains resistant to mercury alone (4 out of 18 compared with 11 out of 133 in strains susceptible to all antibiotics) and strains resistant to all three metals (6 out of 18 compared to 10 out of 133 in strains susceptible to all antibiotics).

Correlation of metal resistance patterns with multiple antibiotic resistance patterns. An alternative approach is to consider the distribution of heavy metals resistance in strains which carry multiple antibiotic resistance markers. The major antibiotic resistance patterns in strains carrying resistance to two or more antibiotics are subdivided into the metal resistance classes in Table 6. In general, as the number of antibiotics to which a strain is resistant increases, the tendency to be multiply resistant to metals also increases. Thus, 5 of the 17 strains resistant to penicillin and erythromycin and 10 out of 87 strains resistant to penicillin and tetracycline were resistant to all three metals, whereas 37 out of 66 strains resistant to

TABLE 5. *Correlation of heavy metal patterns with resistance to penicillin and tetracycline*[a]

Heavy metal resistance			Total set[b]	Single antibiotic resistant subset[c]		
Hg^{2+}	Cu^{2+}	Cd^{2+}		None	Penicillin	Tetracycline
S	S	S	112	36	48	1
R	S	S	57	11	18	4
S	R	S	225	35	153	2
S	S	R	10	2	6	0
R	R	S	37	5	13	2
R	S	R	50	3	9	0
S	R	R	131	31	93	3
R	R	R	82	10	13	6

[a] S, Susceptible; R, resistant.

[b] Total set column describes the metal resistance patterns in the total population of 704 strains studied.

[c] These are a part of the total set and describe the metal resistance patterns for strains resistant to no antibiotics, to penicillin alone, or to tetracycline alone. For example, 353 of the 704 strains studied were resistant to penicillin alone and were distributed among the metal resistance pattern such that 18 of the 353 strains were resistant to mercury only.

TABLE 6. *Correlation of heavy metal resistance patterns with multiple antibiotic resistance patterns*[a]

Heavy metal resistance			Antibiotic resistance patterns			
Hg^{2+}	Cu^{2+}	Cd^{2+}	Pen, Ero	Pen, Tet	Pen, Ero, Tet	Pen, Ero, Tet, Linc, Meth
S	S	S	0	16	7	0
R	S	S	2	13	3	4
S	R	S	5	19	4	0
S	S	R	0	2	0	3
R	R	S	1	11	5	9
R	S	R	1	13	8	6
S	R	R	3	3	2	1
R	R	R	5	10	37	5

[a] Abbreviations: S, susceptible; R, resistant; Pen, penicillin; Ero, erythromycin; Tet, tetracycline; Linc, lincomycin; Meth, methicillin.

penicillin, erythromycin, and tetracycline were resistant to all three metals. Addition of resistance to lincomycin and methicillin reversed this trend and reduced the fraction (5 out of 28) resistant to all three metals.

Metal resistance patterns in coagulase-positive and coagulase-negative strains resistant to a single antibiotic. The strains resistant to no antibiotics, to penicillin, or to tetracycline alone were further subdivided as to their elaboration of coagulase (Table 7). The data are similar to Table 5 with the addition of values from a second series of strains to increase the size of the population studied. In strains with no antibiotic resistance, 82 are coagulase negative and 47 are coagulase positive. This predominance is consistent for each metal resistance pattern, with the exception of the strains resistant to both copper and cadmium which are predominantly coagulase producing. A drastic alteration is found in strains carrying penicillin resistance. The total ratio is now slightly in favor of coagulase-producing strains, largely as a result of the 131 coagulase-producing strains which are resistant to copper alone of the metals. Strains with resistance to cadmium as well as copper counter this trend, with 89 coagulase-negative strains resistant to penicillin, cadmium, and copper, compared with only three coagulase-positive strains. Even with the addition to our study of a second series of strains resistant only to tetracycline, the total number of this class is relatively small. The tetracycline-resistant organisms fall into three major groups: (i) those susceptible to all metals, (ii) those resistant to mercury alone, and (iii) those resistant to all three metals. All of these tetracycline-resistant strains that are also resistant to copper or copper and cadmium are minor classes and possess coagulase activity. All strains possessing mercury resistance and tetracycline resistance are coagulase negative. Therefore, the possession of penicillin resistance changes the distribution of metal resistance patterns and of coagulase production significantly, with an increase in coagulase production and an increase in copper resistance. Introduction of tetracycline resistance in the population, on the other hand, changes the distribution in favor of coagulase-negative strains with resistance to tetracycline. An interesting anomaly occurs in the elaboration of coagulase by strains resistant to copper and cadmium upon the introduction of resistance to penicillin in the population. With no antibiotic resistance present, 28 strains produce coagulase and only three do not. In penicillin-resistant strains, a complete reversal occurs, with only three coagulase producers compared to 89 coagulase-negative strains.

Metal-resistance patterns in coagulase-positive and coagulase-negative strains with resistance to two or more antibiotics. With the exception of one major (28 strains resistant to penicillin, erythromycin, tetracycline, and all three metals) and several minor classes, most of the multiple antibiotic-resistant strains are coagulase negative (Table 8), confirming the observations of others (D. Bentley, personal communication). Although strains resistant to both penicillin and tetracycline are predominantly coagulase negative (80 out of 87), strains resistant to penicillin and erythromycin or resistant to penicillin, erythromycin, and tetracycline are

TABLE 7. *Distribution of resistance to heavy metals in the populations of staphylococci that are resistant only to penicillin and to tetracycline or to no antibiotics, divided with respect to production of coagulase*

Heavy metal resistance[a]			Antibiotic resistance in coagulase-positive and -negative strains					
			None		Penicillin		Tetracycline	
Hg^{2+}	Cu^{2+}	Cd^{2+}	+	−	+	−	+	−
S	S	S	5	31	25	23	0	7
R	S	S	1	10	1	17	0	7
S	R	S	11	20	131	22	3	1
S	S	R	1	1	4	2	0	1
R	R	S	0	5	5	1	0	3
R	S	R	0	3	3	1	0	2
S	R	R	28	3	3	89	3	1
R	R	R	1	9	9	9	0	6

[a] S, Susceptible; R, resistant.

TABLE 8. *Distribution of resistance to heavy metals in the populations of staphylococci resistant to multiple antibiotics, divided with respect to production of coagulase*[a]

Heavy metal resistance[a]			Antibiotic resistance in coagulase-positive and -negative strains							
Hg^{2+}	Cu^{2+}	Cd^{2+}	Pen, Ero		Pen, Tet		Pen, Ero, Tet		Pen, Ero, Tet, Linc, Meth	
			+	−	+	−	+	−	+	−
S	S	S	0	0	0	16	2	5	0	0
R	S	S	0	2	0	13	0	3	0	4
S	R	S	3	2	5	14	3	1	0	0
S	S	R	0	0	0	2	0	0	0	3
R	R	S	0	1	0	11	2	3	0	9
R	S	R	0	1	0	13	2	6	0	6
S	R	R	3	0	1	2	1	1	0	1
R	R	R	4	1	1	9	28	1	0	5

[a] See Table 6 for abbreviation.

usually coagulase positive (Table 8). The lack of coagulase-producing strains resistant to these three antibiotics plus lincomycin and methicillin confirms the scarcity of coagulase-positive, methicillin-resistant strains found in similar populations (D. Bentley, personal communication).

DISCUSSION

Staphylococcus enjoys one of the most successful relationships with man. With current uses of antibiotics and exposure to increased loads of environmental contaminants, the staphylococci inhabiting man have responded by acquiring mechanisms of resistance to each new agent. Exposure to a single antibiotic is known to select strains resistant to several antibiotics (5, 18) and it has been determined that most multiple-resistant isolates of staphylococcus are coagulase negative. Although useful as a routine indicator of pathogenicity, little is known about the genetic determinants of coagulase production or mannitol fermentation. One view is that possession of coagulase is in fact an indicator of species difference in staphylococci. This would suggest the existence of two complete sets of antibiotic- and metal-resistant traits in genetic isolation of each other. Recent studies tend to support the alternate view that staphylococci are a heterogenous group showing a spectrum of characteristics with a degree of genetic exchange. The guanosine plus cytosine ratios of the two types are indistinguishable (15), the tetracycline plasmid for each has similar characteristics (20), interspecific transduction of genetic markers has been demonstrated (31), and pathogenic strains of coagulase-negative bacteria have been placed in biotypes intermediate to *S. aureus* and *S. epidermidis* (29). On the other hand, there is some evidence that little genetic homology exists between coagulase-positive and coagulase-negative strains of staphylococci (N. H. Nielsen, Bacteriol. Proc., p. 45, 1970).

Although it has generally been considered that antibiotic resistance determinants in staphylococci are not genetically linked (17, 23), linkage at the genetic level has been demonstrated in several cases and further circumstantial evidence for the association of various resistance determinants has been demonstrated during this study. A close association between tetracycline and mercury resistance is obvious from an analysis of the patterns in the total population, (Tables 3 and 4). There are two possible approaches to further dissect the relationships described in Tables 3 and 4. One can examine the change in the metal resistance patterns when a single antibiotic resistance is present, compared to the population of antibiotic-susceptible strains. This eliminates the obscuring effects of the interactions of the different antibiotic resistances on the patterns of metal resistance. A second approach to the problem of interpreting the patterns is to observe the change in the heavy metal resistance patterns when a second antibiotic resistance is introduced.

In analysis of the strains resistant to a single antibiotic, penicillin or tetracycline, as compared to the strains susceptible to all antibiotics tested (Table 5), the association between resistance to copper and penicillin was detected. Resistance to multiple antibiotics resulted in a general tendency to be resistant to multiple heavy metals. Dissection of the metal resistance patterns for single antibiotic-resistant strains into coagulase-negative and coagulase-producing strains demonstrated the general association of resistance to mercury and tetracycline

with lack of coagulase production, whereas resistance to copper, penicillin, and production of coagulase were associated (Table 7). Strains resistant to many agents were usually coagulase negative. For example, in the strains resistant to penicillin, erythromycin, tetracycline, copper, mercury, and cadmium, 28 were coagulase-negative and only one was coagulase-positive (Table 8).

Knowledge of the associations between antibiotic and heavy metal resistance might well prove valuable in two ways. First, the association of pathogenic characters and heavy metal resistance could add to the already serious problems of the management of infections due to the close association of rapidly transferable resistance to several antibiotics for some microorganisms. Second, the perturbations of the normal patterns of distributions of heavy metal resistance in microorganisms inhabiting man could serve as an assay for heavy metal contamination. An attempt has been made to determine the effect of heavy metal loads in changing these patterns with microorganisms isolated from populations "at risk" from heavy metal exposure, as well as "normal" control populations (12).

Now that the epidemiologic patterns have been identified in clinical isolates, further studies utilizing deoxyribonucleic acid-mediated transformation are in progress to determine the molecular bases for the interspecific and intraspecific relationships between determinants for antibiotic and heavy metal resistance in the staphylococci described in this study.

ACKNOWLEDGMENTS

We would like to thank David L. Hollis and Judy Fitzgerald for expert technical assistance. We would also like to thank Helen Short for valuable assistance and discussions.

This investigation was supported in part by a National Science Foundation (RANN) grant no. GI-30097.

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