The Journal of Medical Microbiology

Vol. 13, No. 3

RESISTANCE TO CADMIUM, ARSENATE AND MERCURY AMONG DANISH STRAINS OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CASES OF BACTERIAEMIA, 1957–74

VIBEKE T. ROSDAHL AND KIRSTEN ROSENDAL*

The Institute of Medical Microbiology, Department of Clinical Bacteriology, University of Copenhagen, Juliane Mariesvej 22, DK-2100 Copenhagen, and *The Department of Hospital Infections, State Serum Institute, Amager Boulevard 80, DK-2300 Copenhagen, Denmark

ENDEMIC hospital strains of *Staphylococcus aureus* are often resistant to mercury (Hg) ions (Moore, 1960). Determinants for resistance to Hg and other heavy-metal ions, such as cadmium (Cd) and arsenate (As), are often on the penicillase plasmid (Richmond and John, 1964; Dyke, Parker and Richmond, 1970) and strains resistant to all three metals often have multiple resistance to antibiotics (Dyke *et al.*, 1970; Nakahara *et al.*, 1977) and produce large amounts of penicillinase (Richmond *et al.*, 1964).

Different penicillinase plasmids harbour different heavy-metal resistances (Novick and Richmond, 1965; Dyke *et al.*, 1970; Lacey and Rosdahl, 1974), and changes in metal-resistance pattern might be expected to reflect changes in the occurrence of plasmids in *S. aureus*. So far, investigations of metal resistance have been performed only on strains isolated during a short period or from a few hospitals (Dyke *et al.*, 1970; Nakahara *et al.*, 1977). The investigations described here were performed on bacteriaemia strains that were epidemiologically unrelated (Jessen *et al.*, 1969) and had been isolated during a long period, 1957–74 (Rosendal *et al.*, 1976b).

MATERIALS AND METHODS

Staphylococci. Since 1957 the Department of Hospital Infections at the State Serum Institute, Copenhagen has collected strains of *S. aureus* isolated from blood cultures from hospitals all over Denmark (Jessen *et al.*, 1969; Rosendal *et al.*, 1976b). In the present study we examined 3240 of these strains; these comprised about one-half of those isolated in 1957–58, randomly selected (91 strains), and all of those isolated in 1967 (398 strains), 1969 (473 strains), 1971 (542 strains), 1972 (609 strains), 1973 (611 strains) and 1974 (516 strains). In addition, 101 strains of

---

Received 8 May 1979; revised version accepted 4 Sept. 1979.

phage type 80/81 and 66 strains with the phage-typing pattern 52/52A/80/81 or 52/52A/80 (isolated during 1960–66), and 33 strains of the 94, 96 complex (isolated during 1976–77) were included in special examinations. Since the year of isolation all strains had been kept in the freeze-dried state; to ensure identity between the original strains and the freeze-dried ones, all freeze-dried strains were tested for their sensitivity to several antibiotics; only strains with their original sensitivity patterns were included in the present investigation.

Methods. During the year of isolation or soon afterwards, all strains were examined for resistance to antibiotics and were phage typed (Rosendal *et al.*, 1976a). The subdivision into phage groups and complexes was done according to Parker (1962). All the freeze-dried strains chosen for study were transferred to nutrient broth, and the resistance to penicillin G (P), streptomycin (S), chloramphenicol (C), tetracycline (T), erythromycin (E) and fusidic acid (F) was determined by the Ericsson disc method (Ericsson and Sherris, 1971). Resistance to methicillin (M) was performed as described by Eriksen and Erichsen (1964).

The minimum inhibitory concentrations (MIC) of Cd, As and Hg for the strains were determined on agar plates with four different concentrations of each salt. Each strain was streaked on all 12 plates plus a control plate not containing heavy-metal ions, and growth was read after overnight incubation. Strains for which the MIC of Cd was cadmium acetate ≥ 27 mg/litre ($\equiv 10^{-4}$ M), of As was sodium arsenate ≥ 400 mg/litre and of Hg was mercuric chloride ≥ 50 mg/litre were regarded as resistant.

RESULTS

Strains excluded from the investigation

Of the 3240 strains, 25 could not be re-examined for various technical reasons; of the remaining 3215, 175 (5%) appeared to have a resistance pattern different from that found in the year of isolation and were also excluded. The percentage of strains excluded was fairly constant (3–9%) during the years of investigation. All the 116 strains that had lost their P resistance were further examined, and it was found that 56% of them were originally resistant to P only, whereas 31% had multiple resistance to P, S, T and possibly more antibiotics (PST $\pm$). The percentage of these two resistance patterns in the whole material were 45 and 26 respectively.

Patterns of metal resistance

Strains with all possible patterns of resistance and sensitivity to the three metals occurred, but the four most common were: resistant to Cd and As (Cd,As), 32%; resistant to all three metals (Cd,As,Hg), 27%; resistant to Cd only (Cd), 25%; and sensitive to all three metals (none), 14%. Strains exhibiting other resistance patterns comprised less than 3% of the total.

There were only small changes in the patterns from 1957–58 to 1967 (table I); therefore the material from the intervening years was not examined. From 1967 to 1974 the percentage of Cd strains increased from 18 to 32 and the percentage of Cd,As strains from 23 to 33, but the percentage of Cd,As,Hg strains fell from 48 to 13.

Relationship of metal resistance to antibiotic resistance

Of the strains sensitive to all metal ions, 44% were also sensitive to all

TABLE I

Patterns of metal resistance in 3040 strains of Staphylococcus aureus isolated in selected years from 1957-58 to 1974

Year of isolation (and number) of strains tested	Percentage of strains with metal-resistance pattern:							
	none	Cd	As	Hg	Cd,As	Cd,Hg	As,Hg	Cd,As,Hg
1957-58 (84)	11	11	1	0	26	1	0	50
1967 (380)	6	18	1	0	23	3	0	48
1969 (451)	8	20	<1	<1	30	2	0	39
1971 (503)	14	24	<1	<1	32	<1	0	28
1972 (550)	23	24	<1	<1	30	0	<1	22
1973 (589)	10	32	1	0	39	<1	0	18
1974 (483)	19	32	2	0	33	<1	0	13

Cd=resistance to cadmium, As=resistance to arsenate, Hg=resistance to mercury, and to combinations of these metals as indicated. None=no resistance, i.e., sensitive to all these metals.

antibiotics, whereas 46% were P resistant only (table II). Among the strains resistant to Cd only, 15% were antibiotic sensitive, but as many as 76% were P resistant only. Of the strains resistant to Cd,As, 31% were antibiotic sensitive and 56% were P resistant only. The strains that were PST± resistant amounted to 83% of the Cd,As,Hg-resistant strains. Apart from the fact that nearly all Hg-resistant strains were resistant to P either alone or in combinations with other antibiotics (only five out of 829 Hg-resistant strains were sensitive to P), no clear-cut correlation could be found between resistance to antibiotics and metal resistance.

TABLE II

Relationship of metal-resistance and antibiotic-resistance patterns in 2956 strains of Staphylococcus aureus isolated during the selected years from 1967 to 1974

Metal-resistance pattern (and number of strains)	Percentage (from total number tested) of strains with antibiotic-resistance pattern:					
	sens. to all (589)	P (1341)	PS (145)	PT (76)	PST± (735)	other (70)
None (407)	44	46	2	3	2	3
Cd (757)	15	76	2	4	<1	3
Cd,As (938)	31	56	5	1	4	2
Cd,As,Hg (792)	<1	4	9	2	83	1
Other (62)	19	34	8	0	36	3

Sens. to all antibiotics=sensitive to penicillin (P), streptomycin (S), chloramphenicol, tetracyclines (T), erythromycin, and methicillin; P=resistant to P only; PS=resistant to PS only; PT=resistant to PT only; PST±=resistant to PST with or without resistance to other antibiotics; other=resistant to other antibiotics or combinations of antibiotics.

Other abbreviations as in table I.

TABLE III

Relationship of metal resistance to phage type among strains of Staphylococcus aureus with different antibiotic-resistance patterns*

Antibiotic resistance	Phage group or complex	Number of strains	Percentage of strains with metal resistance:				
			none	Cd	Cd,As	Cd,As,Hg	other
Sensitive	52,52A,80,81†	71	18	0	80	0	1
	Group I	128	14	12	74	0	0
	Group II	90	41	12	44	0	2
	Group III	106	46	35	15	<1	3
	83A	36	42	22	28	0	8
	94,96	4	(75)	(25)	0	0	0
	Other‡	154	28	25	44	1	2
	Total	589	30	19	49	<1	2
Resistant to P only	52,52A,80,81†	72	4	6	68	22	0
	Group I	251	3	14	78	3	2
	Group II	265	25	18	55	<1	2
	Group III	358	13	71	13	1	1
	83A	122	15	72	11	<1	2
	94,96	49	24	76	0	0	0
	Other‡	224	14	47	35	<1	3
	Total	1341	14	43	39	2	2
Resistant to PS only	52,52A,80,81†	95	1	0	33	63	3
	Group I	10	0	0	(50)	(50)	0
	Group II	1	0	0	(100)	0	0
	Group III	19	(16)	(26)	(48)	(11)	0
	83A	5	0	(20)	(20)	(40)	(20)
	94,96	2	(50)	(50)	0	0	0
	Other‡	13	(23)	(46)	(8)	(15)	(8)
	Total	145	6	9	33	49	4
Resistant to PST ±	52,52A,80,81†	34	0	0	26	68	6
	Group I	2	0	0	0	(100)	0
	Group II	5	0	(20)	(80)	0	0
	Group III	72	3	4	4	86	3
	83A	603	1	0	4	92	3
	94,96	1	0	(100)	0	0	0
	Other‡	18	0	0	(6)	(89)	(6)
	Total	735	1	<1	6	90	3

Abbreviations as in tables I and II.

* In 2810 strains from the selected years 1967–74 (table I); 76 strains resistant to PT only and 70 strains resistant to combinations of antibiotics other than those referred to are not included.

† See Asheshov and Winkler (1966).

‡ Non-typable strains and strains referred to as "miscellaneous".

Figures in parentheses indicate percentages derived from numbers of strains that were too small for valid calculation.

Relationship between phage type, metal resistance and antibiotic resistance

The four dominant metal-resistance patterns of the various phage groups and complexes are shown in table III. The various phage groups and complexes were found to have characteristic metal-resistance patterns. Among antibiotic-sensitive strains and strains resistant to P only, strains of the 52, 52A, 80, 81 complex and the closely related group-I strains had a high percentage of As-resistant strains and a low percentage of strains sensitive to all three metal ions, whereas the opposite proportion was found among strains of group III and the 83A complex. Group II had a high percentage of metal-sensitive strains and had a higher percentage of As-resistant strains than was found among group-III strains.

Strains resistant to PS were predominantly members of the 52, 52A, 80, 81 complex, and strains with multiple resistance were predominantly members of the 83A complex. In both groups the percentage of strains resistant to Cd, As, Hg was very high.

More investigations were made to determine whether any phage type was characterised by a particular pattern of metal resistance. This seemed to be the case with type 80/81, because 26 out of 28 strains investigated—irrespective of antibiotic sensitivity—were resistant to Cd, As, Hg, whereas none of the other types within the complex (seven type 80, 19 type 81, 149 type 52/52A/80/81 or 52/52A/80, and 69 of other phage patterns) showed any deviation from the common metal-resistance pattern of the whole complex. Thus the percentage of strains resistant to all three metal ions was found to be much higher in type 80/81 than in the closely related type 52/52A/80/81; none of either type was resistant to Cd alone. In contrast, none of the strains of the 94, 96 complex was resistant to As and Hg, whereas 81% were resistant to Cd alone.

DISCUSSION

Our results show that the distribution of resistance to Cd, As and Hg among strains of *S. aureus* was not random; 98% of all the strains examined had one of four patterns of resistance: (1) sensitive to all these metals, (2) resistant to Cd, (3) resistant to Cd, As and (4) resistant to Cd, As, Hg. During the period of study, changes in the relative proportions of strains with these four patterns of metal resistance could be correlated with alterations in antibiotic-resistance and phage-typing patterns of the prevalent strains (Rosendal *et al.*, 1976b). Because the determinants for metal resistance are often, but not always, on the penicillinase plasmid (Novick and Roth, 1968; Dyke *et al.*, 1970; Kondo, Ishikawa and Nakahara, 1976), the occurrence of different types of penicillinase plasmid may have changed gradually during the years 1957–74. It is improbable that these changes are restricted to strains isolated in Denmark, but it is difficult to compare the results of other authors with ours because some included a higher percentage of multiply-resistant strains (Nakahara *et al.*, 1977) or strains collected during one year from a few hospitals (Dyke *et al.*, 1970). However, it is remarkable that none of the

English strains was resistant to Cd alone, whereas 11–18% of the Danish strains had this resistance pattern.

The reason for the dominance of the four metal-resistance patterns is uncertain. It is not known whether other combinations of resistance are less stable in staphylococcal strains. Of the 64 strains that had other metal-resistance patterns, 28 were resistant to As only; 46% of these strains were antibiotic sensitive and 39% resistant only to P, a distribution similar to that found in Cd,As strains. Furthermore, of the 30 Cd,Hg-resistant strains, 60% had multiple antibiotic resistance, thus resembling the Cd,As,Hg-resistant strains. This suggests that the As-resistant strains may be Cd,As-resistant strains that have lost Cd resistance and the Cd,Hg strains are derived from Cd,As,Hg-resistant strains by loss of As resistance. Three of the Cd,Hg-resistant strains were resistant to F, and were shown to lose F resistance together with P resistance, so it is possible that the gene for F resistance was originally chromosomal and was transferred to the Cd,As,Hg-resistance penicillinase plasmid, excluding As at the time of insertion.

It has not previously been observed that strains resistant to Cd alone are mainly resistant to P only and only 15% are antibiotic sensitive, but the correlation between multiple antibiotic resistance and Cd,As,Hg resistance agrees with the results of Dyke *et al.* (1970) and Nakahara *et al.* (1977). In strains with this antibiotic-resistance pattern there seems to be a close connection between P resistance and resistance to Cd,As,Hg. Among the 116 strains that lost their P resistance during the storage, 35 were originally multiply resistant to antibiotics and of these, 28 were sensitive to the metals, and the remaining seven were resistant to only one or two of the metals after storage. Unfortunately we do not know the Cd, As resistance of these strains at the time of isolation; however, Hg resistance had been determined by the method of Moore (1960), and because determinations by this method correspond in 98% of cases with the MIC determinations, 30 of the 36 strains were probably Hg resistant at the time of isolation. Because 90% of the multiply-resistant strains were Cd,As,Hg resistant, the loss of penicillin resistance is probably accompanied by a simultaneous loss of resistance to Cd,As,Hg.

This connection between P resistance and resistance to Cd,As,Hg is found almost exclusively in strains that are PST $\pm$ or PS resistant and not in strains that are resistant to P alone because only 2% of these strains, mainly from the 52, 52A, 80,81 complex and group I, were resistant to Cd,As,Hg. Among the 116 strains that lost their P resistance on storage in the laboratory, 82 were P resistant only at the time of isolation and only one of them was Hg resistant. Thus no conclusion can be drawn about the connection between heavy-metal resistance and P resistance in these strains. More investigations should be made into the connection between different metal-resistance patterns and the type and the amount of the penicillinase enzyme produced.

The correlation between phage type and Cd,As,Hg resistance is very much influenced by the correlation between phage type and antibiotic resistance. Strains belonging to the 52,52A,80,81 and the 94,96 complexes show a remarkable correlation between phage-typing pattern and metal-resistance pattern

TABLE IV

Metal and antibiotic resistance in strains of Staphylococcus aureus of phage types 80/81 and 52/52A/80/81*

Phage type	Antibiotic resistance: and number of strains	Percentage of strains with metal-resistance				
		none	Cd	Cd,As	Cd,As,Hg	other
80/81	Sensitive: 4	(25)	0	(75)	0	0
	P: 19	0	0	(11)	(89)	0
	PS: 82	0	0	5	95	0
	PST ±: 22	0	0	5	95	0
	other: 2	(50)	0	(50)	0	0
	Total 129	2	0	9	90	0
52/52A/80/81 or 52/52A/80	Sensitive: 33	15	0	85	0	0
	P: 31	0	0	68	32	0
	PS: 134	<1	0	31	69	0
	PST ±: 13	0	0	(46)	(38)	(15)
	Other: 4	(75)	0	0	(25)	0
	Total 215	4	0	45	50	<1

Abbreviations as in tables I and II.

* The material included 28 strains of phage type 80/81 and 149 of type 52/52A/80/81 or 52/52A/80 isolated during 1967-74; and 101 of type 80/81 and 66 of type 52/52A/80/81 or 52/52A/80 isolated during 1960-66.

Figures in parentheses indicate percentages derived from numbers of strains too small for valid calculation.

TABLE V

Metal and antibiotic resistance in strains of Staphylococcus aureus belonging to the phage complex 94, 96*

Antibiotic resistance: and number of strains	Percentage of strains with metal-resistance†:	
	none	Cd
Sensitive: 6	(33)	(67)
P: 83	12	88
Other: 14	(57)	(43)
Total 103	19	81

Abbreviations as in tables I and II.

* The material consisted of 70 strains isolated in 1972, 1973 and 1974 and 33 in 1976 and 1977.

† None of the strains was resistant to Cd,As or Cd,As,Hg.

Figures in parentheses indicate percentages derived from numbers of strains that were too small for valid calculation.

irrespective of antibiotic resistance. Among strains of the 52, 52A, 80, 81 complex (Asheshov and Winkler, 1966), only about 1% were resistant to Cd alone (table III), and of the strains with phage-type 80/81 and 52/52A/80/81 none was Cd resistant only. The majority of these strains were PS resistant and, as shown in table IV, 90% of strains of phage type 80/81 and 50% of type 52/52A/80/81 had the metal-resistance pattern Cd,As,Hg. This is in good agreement with the results of Moore (1960) and Green (1962), who studied Hg resistance only and found that 91–95% of the strains belonging to the 52, 52A, 80, 81 complex were Hg resistant. Apparently these strains often harbour plasmids for Cd,As,Hg, or less often Cd,As resistance, but they never harbour Cd-resistance plasmids. It is also notable that strains of phage-type 52/52A/80/81 more often contain Cd,As-resistance plasmids than do the strains of phage type 80/81. These differences might be explained either by the failure of a strain to accept a certain plasmid or by a process of strain selection.

Strains of phage type 94/96 or of the phage complex 94, 96 were either fully sensitive or Cd resistant but they never had the metal-resistance patterns Cd,As or Cd,As,Hg (table V). Whether this is due to an intrinsic lack in the capacity of the strains to harbour these plasmids or whether it is due to selection is uncertain. Strains of the 94, 96 complex seem, in our hands, to be related to group-II strains but they are never lysed by phage 3A when tested with phages in concentrations above RTD; among group-II strains, type 3A is the only one that had a higher percentage of strains with the metal-resistance pattern Cd,As.

By investigating the strains that had lost one or several of the resistance properties found in the year of isolation, we were able to get a rough picture of the connections between the different antibiotic-resistance genes. Among the 116 strains that lost their P resistance, 20 strains were E resistant originally, but only one simultaneously lost E and P resistance; one of 41 S-resistant strains showed a simultaneous loss of S and P resistance. These observations do not suggest any close connection between P resistance and E or S resistance and neither T or M resistance was found to be associated with P resistance.

SUMMARY

A collection of 3040 epidemiologically-unrelated strains of *Staphylococcus aureus* isolated from blood cultures during the period 1957–74 was examined for their resistance to cadmium (Cd), arsenate (As) and mercury (Hg); 98% of the strains had metal-resistance patterns of (a) sensitive to all these metals, (b) resistant to Cd, (c) resistant to Cd,As or (d) resistant to Cd,As,Hg. The percentage of Cd,As,Hg-resistant strains fell from 50 to 13 in the period of investigation and the percentage of Cd-resistant strains increased from 11 to 32. Strains resistant only to Cd were mainly P resistant only and a correlation was found between multiple antibiotic resistance and Cd,As,Hg resistance. Strains of phage types 80/81, 52/52A/80/81 and 52/52A/80 were never Cd resistant only, but mainly Cd,As,Hg resistant. Strains of the 94, 96 complex were mainly Cd resistant and were never resistant to Cd,As,Hg.

We thank Inger Gasbjerg and Dorte Østergaard at the Institute of Medical Microbiology and Jytte Mark and Lis Monies at the State Serum Institute for valuable help and skilful technical assistance.

REFERENCES

- ASHESHOV, E. H. AND WINKLER, K. C. 1966. *Staphylococcus aureus* strains in the "52/52A/80/81 complex". *Nature, Lond.*, **209**, 638.
- DYKE, K. G. H., PARKER, M. T. AND RICHMOND, M. H. 1970. Penicillinase production and metal-ion resistance in *Staphylococcus aureus* cultures isolated from hospital patients. *J. med. Microbiol.*, **3**, 125.
- ERICSSON, H. M. AND SHERRIS, J. C. 1971. Antibiotic sensitivity testing. Report of an international collaborative study. *Acta path. microbiol. scand.*, sect. B, suppl., **217**.
- ERIKSEN, K. R. AND ERICHSEN, I. 1964. Resistance to methicillin, isoxazolyl penicillins and cephalothin in *Staphylococcus aureus*. *Acta path. microbiol. scand.*, **62**, 255.
- GREEN, S. M. 1962. Mercury sensitivity of staphylococci. *J. clin. Path.*, **15**, 249.
- JESSEN, O., ROSENDAL, K., BÜLOW, P., FABER, V. AND ERIKSEN, K. R. 1969. Changing staphylococci and staphylococcal infections. A ten year study of bacteria and cases of bacteraemia. *New Engl. J. Med.*, **281**, 627.
- KONDO, I., ISHIKAWA, T. AND NAKAHARA, H. 1974. Mercury and cadmium resistances mediated by the penicillinase plasmid in *Staphylococcus aureus*. *J. Bact.*, **117**, 1.
- KONDO, I., ISHIKAWA, T. AND NAKAHARA, H. 1976. Heavy metal resistance in *Staphylococcus aureus*. *Zentbl. Bakt. ParasitKde*, I. Abt. Orig., suppl., **5**, 217.
- LACEY, R. W. AND ROSDAHL, V. T. 1974. An unusual "penicillinase plasmid" in *Staphylococcus aureus*; evidence for its transfer under natural conditions. *J. med. Microbiol.*, **7**, 1.
- MOORE, B. 1960. A new screen test and selective medium for the rapid detection of epidemic strains of *Staph. aureus*. *Lancet*, **2**, 453.
- NAKAHARA, H., ISHIKAWA, T., SARAI, Y. AND KONDO, I. 1977. Distribution of resistances to metals and antibiotics of staphylococcal strains in Japan. *Zentbl. Bakt. ParasitKde*, I. Abt. Orig., **A237**, 470.
- NOVICK, R. P. AND RICHMOND, M. H. 1965. Nature and interaction of the genetic elements governing penicillinase synthesis in *Staphylococcus aureus*. *J. Bact.*, **90**, 467.
- NOVICK, R. P. AND ROTH, C. 1968. Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. *J. Bact.*, **95**, 1335.
- PARKER, M. T. 1962. Phage-typing and the epidemiology of *Staphylococcus aureus* infection. *J. appl. Bact.*, **25**, 389.
- RICHMOND, M. H. AND JOHN, M. 1964. Co-transduction by a staphylococcal phage of the genes responsible for penicillinase synthesis and resistance to mercury salts. *Nature, Lond.*, **202**, 1360.
- RICHMOND, M. H., PARKER, M. T., JEVONS, M. P. AND JOHN, M. 1964. High penicillinase production correlated with multiple antibiotic resistance in *Staphylococcus aureus*. *Lancet*, **1**, 293.
- ROSENDAL, K., BÜLOW, P., BENTZON, M. W. AND ERIKSEN, K. R. 1976a. *Staphylococcus aureus* strains isolated in Danish hospitals from January 1st 1966 to December 31st 1974. *Acta path. microbiol. scand.*, sect. B., **84**, 359.
- ROSENDAL, K., JESSEN, O., BÜLOW, P. AND FABER, V. 1976b. Reduction of multi-resistant staphylococci in Danish hospitals 1967-1974. *Zentbl. Bakt. ParasitKde*, I. Abt. Orig., suppl., **5**, 1027.