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Pattern of antibiotic and heavy-metal ion resistance in recent hospital isolates of *Staphylococcus aureus*

By M. R. MILLAR, N. GRIFFIN and N. KEYWORTH
Departments of Medical Microbiology and Pathology, Leeds General Infirmary

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SUMMARY

Two hundred and one strains of *Staphylococcus aureus* isolated from in-patients and out-patients were examined for sensitivity to antibiotics, heavy-metal ions and ethidium bromide and for phage-typing pattern.

Heavy-metal ion resistance was less frequent than reported in previous studies and was as frequent in penicillinase non-producing as producing strains. 'Methicillin-resistant' strains were resistance to ethidium bromide and mercury. Resistance to heavy-metal ions, including cadmium, may be becoming less common amongst clinical isolates of *S. aureus*.

INTRODUCTION

Plasmids mediating penicillinase production by *Staphylococcus aureus* frequently carry determinants for resistance to heavy-metal ions such as cadmium, arsenate and mercury (Richmond & John, 1964; Novick & Roth, 1968). Resistance to all three metal ions may be associated with multiple antibiotic resistance (Dyke, Parker & Richmond, 1970; Nakahara *et al.* 1977). The high frequency of heavy-metal ion resistance in *S. aureus* may follow co-selection of heavy-metal ion resistance with penicillin resistance as a result of penicillin therapy. Alternatively it has been suggested that environmental cadmium may select for resistant strains (Kondo, Ishikawa & Nakahara, 1974). Rosdahl & Rosendal (1980) described a fall in the frequency of heavy-metal ion resistance in Danish strains of *S. aureus* between 1957 and 1974, but there are no recent reports from the United Kingdom of the frequency of heavy-metal ion resistance in hospital isolates of *S. aureus*. This study reports the frequency of heavy-metal ion resistance and of resistance to antibiotics in recent hospital isolates from the Leeds General Infirmary.

MATERIALS AND METHODS

Strains of Staphylococcus aureus

Two hundred and one separate strains were isolated from patients attending the Leeds General Infirmary as in-patients or out-patients between December 1985 and February 1986. All of the strains were stored on nutrient agar slopes at room temperature for up to 3 months prior to testing for susceptibility to antibiotics and heavy-metal ions. Multiple isolates from the same patient and of the same phage type were included as one isolate.

Identification of Staphylococcus aureus

All of the strains included in this study gave a positive tube coagulase (Cowan, 1938) and DNase (DiSalvo, 1958) test.

Susceptibility to antibiotics and heavy-metal ions

Susceptibility was determined using the disk diffusion method of Stokes & Ridgway (1980) to penicillin 1 μg, erythromycin 10 μg, tetracycline 10 μg, gentamicin 10 μg, chloramphenicol 10 μg, sodium fusidate 10 μg, cloxacillin 2·5 μg and cephradine 30 μg. Strains of *S. aureus* were defined as sensitive if the zone radius was equal to or within 2 mm smaller than that of *S. aureus* strain Oxford (NCTC 6571). Methicillin resistance was determined by disk susceptibility testing at 30 °C on Iso-sensitest agar (Oxoid Limited, Hampshire).

The minimum inhibitory concentration (MIC) for each strain was determined by inoculation of 10^4 viable cells onto Iso-sensitest agar containing doubling concentrations of cadmium acetate from 10^{-5} to 10^{-2} M, mercuric chloride from 10^{-6} to 10^{-4} M or sodium arsenate from 5×10^{-5} to 10^{-2} M. *S. aureus* strains Oxford and 13136 (Jevons, 1961) were used as heavy-metal ion sensitive and resistant controls.

Phage typing

The majority of *S. aureus* isolates from hospital patients were phage-typed as a routine procedure by a modification of the method of Blair & Williams (1961). Five ml of peptone water was inoculated with the strain to be tested and incubated for 18 h at 37 °C without shaking. The peptone water culture was used to flood nutrient agar plates containing 5·5 mM calcium chloride. These plates were drained and dried before application of phage preparations at RTD × 100 and then were incubated at 30 °C for 18 h. Reactions were scored with + = 20–50 plaques and + + = > 50 plaques.

RESULTS

Minimum inhibitory concentrations were bimodal for sodium arsenate and mercuric chloride and trimodal for cadmium acetate. Resistance was defined as an MIC exceeding 10^{-4} M for low level and 10^{-3} for high level cadmium resistance, $2{\cdot}2 \times 10^{-5}$ M for mercuric chloride and $1{\cdot}28 \times 10^{-3}$ M for sodium arsenate. The MICs for resistant strains ranged from 10^{-4} to 5×10^{-3} M for cadmium acetate, $3{\cdot}2 \times 10^{-5}$ to $1{\cdot}3 \times 10^{-4}$ for mercuric chloride and sodium arsenate from $3{\cdot}2 \times 10^{-3}$ to 10^{-2} M. Sensitive strains were inhibited by concentrations of 2×10^{-5} to 8×10^{-5} M for cadmium acetate, 4×10^{-6} to $1{\cdot}6 \times 10^{-5}$ M for mercuric chloride and 10^{-4} to 8×10^{-4} M for sodium arsenate. Of the 201 strains studied, 179 (89%) were penicillin resistant (Table 1), but resistance to other antibiotics was less common. Cadmium resistance was predominantly high level (MIC of $< 10^{-3}$ M). Cadmium resistance was as common amongst penicillin sensitive as resistant strains. Ninety-six (47·8%) of the 201 strains were resistant to penicillin alone compared to 84 (41·8%) resistant to both penicillin and cadmium acetate (10^{-4} M). Seventeen (8·5%) strains were resistant to cadmium (10^{-4} M) but sensitive to penicillin. Mercury resistance was uncommon but the 9 mercury resistant strains included 2

Table 1. *Percentage of hospital isolates resistant to antibiotics and heavy-metal ions*

Resistant to	Hospital isolates (%) ($n = 201$)
Penicillin	89·6
Erythromycin	10·9
Tetracycline	3·5
Chloramphenicol	0·0
Gentamicin	5·5
Sodium fusidate	5·5
Cephradine*	4·0
Cloxacillin†	0·0
Cadmium (10^{-4} M)	4·9
Cadmium (10^{-3} M)	45·3
Mercury	4·5
Arsenate	46·8
Ethidium bromide	5·0

* Including seven 'methicillin-resistant' strains and one strain showing resistance to penicillin and cephradine.

† All isolates including 'methicillin-resistant' strains were sensitive to cloxacillin by disc testing at 37 °C.

resistant to penicillin, erythromycin and tetracycline and 7 'methicillin-resistant' strains (MRSA). Six of the seven MRSA strains were resistant to erythromycin, gentamicin, sodium fusidate, cadmium and mercury. One MRSA strain was also arsenate resistant.

One hundred and eighty-five of the 201 (92%) isolates were phage-typed. Thirty-eight strains (20·5%) gave reactions in more than one phage group, 67 (36·2%) gave reactions in one phage group only and 80 (43·2%) strains were not typable (NT) using the current range of phages at RTD × 100. No strain was resistant to cadmium or arsenate or mercury alone, but resistance to penicillin alone was frequent. This pattern was found with a frequency varying from 12·8% in strains with phage group 1 reactions to 52·5% in NT strains. The only other pattern of resistance which was frequent was resistance to penicillin and cadmium and arsenate, which was found in 71·08% of strains with phage group 1 reactions, but in only 18·8% of NT strains. Only ten strains were resistant to ethidium bromide, including MRSA strains and three NT isolates.

DISCUSSION

In several studies, the frequency of cadmium resistance in strains of *S. aureus* has been reported to exceed 80% (Dyke, Parker & Richmond, 1970; Kondo, Ishikawa & Nakahara, 1974; Rosdahl & Rosendal, 1980). Explanations for the high frequency of cadmium resistance include co-selection of cadmium resistance by penicillin therapy because determinants for penicillinase synthesis are usually found between genes coding for cadmium and arsenate resistance in penicillinase plasmids (Novick *et al.* 1979; Shalita, Murphy & Novick, 1980). Another explanation involves selection of cadmium resistance by environmental cadmium. Heavy-metal ions are common environmental pollutants. There is a

high content of cadmium in cigarette smoke (Shroeder, Balassa & Hogencamp, 1961; Nandi *et al.* 1969) and cadmium accumulates in tissues with time (Lewis, Jusko & Caughlin, 1972). The amount of cadmium that adsorbs to nasal hair and epithelial lining in smokers has not been measured, but in this study cadmium-sensitive strains of *S. aureus* were inhibited by cadmium concentrations as low as 2×10^{-5} M.

In this study, penicillin resistance, not associated with heavy-metal ion resistance, was the commonest resistance pattern. Although associated with multiple antibiotic resistance, as previously reported (Richmond & John, 1964), mercury resistance was uncommon and resistance to heavy-metal ions including cadmium was no more common in penicillin resistant than sensitive strains. The findings of this study may reflect the present frequency of cadmium resistance in other areas of the United Kingdom. Ethidium bromide resistance has been reported as a characteristic of Australian MRSA strains (Townsend, Ashdown & Grubb, 1985), but was found in the seven MRSA strains included in this study. Six of the seven MRSA strains showed cadmium and mercury resistance, with arsenate sensitivity, which is a pattern of resistance also reported in Australian MRSA strains (Grubb *et al.* 1983). Townsend *et al.* (1987) have recently reported London hospital isolates of *S. aureus* with similar characteristics to Australian MRSA strains.

Although the reduced frequency of cadmium resistance reported in this study may be a result of reduced levels of environmental cadmium and a reduction in the incidence of smoking, a more likely explanation is that *S. aureus* is evolving such that plasmids coding for both penicillin and heavy-metal ion resistance are becoming less frequent. In support of this hypothesis is the finding that cadmium resistance was no more common in penicillin-resistant that -sensitive strains.

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