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Metal resistance in *Acinetobacter* and its relation to β-lactamase production

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Thirty nine clinical isolates of *Acinetobacter* belonging to six species were tested for resistance to 20 metal ions and their ability to produce β-lactamase. Fifty two percent of the strains produced β-lactamase. β-Lactamase producers and non-producers were almost equally distributed in the different species. *A. baumannii* was the predominant biotype and was found to be most resistant to metals. Resistance to mercury was prevalent in β-lactamase-producing *A. baumannii* only. Silver resistant strains of *A. baumannii* produced β-lactamase. Sensitivity and resistance to copper and cadium was equally distributed between β-lactamase producers and non-producers. β-Lactamase-producer and -non-producer strains were uniformly sensitive to cadmium except *Acinetobacter* genospecies 1.

Keywords: *Acinetobacter*, β-lactamase, metal resistance

Introduction

Environments such as waste water and water bodies are frequently contaminated with a variety of toxic metal ions. High levels of multiple metal resistant bacteria have been isolated from environments contaminated with toxic metals (Summers & Jacoby 1978). These resistant phenotypes are maintained in the gene pool by exposure of the bacteria to the metal ions in the environment (Trevors *et al.* 1985, Towner 1991). Silver, copper and bismuth are widely used in chemotherapy (Wysor 1972, Sox *et al.* 1989); hence these are present in the hospital environment. Data on bacterial metal resistance has been compiled (Trevors *et al.* 1985), but such information about *Acinetobacter* has only recently become available (Neito *et al.* 1989). We have reported metal resistance in *Acinetobacter* strains isolated from clinical as well as environmental sources (Chinchalkar *et al.* 1990).

Acinetobacter, a Gram-negative coccobacillus, is ubiquitous in nature. Moreover, it is regarded as one of the most resistant nosocomial pathogens (Larson 1984; Chopade 1986). There is an increased isolation of β-lactam resistant *Acinetobacter* strains. This resistance probably results from the production of β-lactamase (Goldstein *et al.* 1983). Mercury and cadmium resistance is often linked to β-lactamase production in staphylococcal plasmids (Moore 1960, Richmond & John 1964, Novick & Roth, 1968, Shales *et al.* 1991). The correlation between metal resistance and β-lactamase production in *Branhamella* has been used by Riley *et al.* (1989) in an attempt to develop a typing scheme. In the present study we have studied the metal resistance of *Acinetobacter* species with respect to their ability to produce β-lactamase.

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Materials and methods

Bacterial strains

A total of 39 clinical isolates of *Acinetobacter* from blood, urine and pus samples isolated from local hospitals in Pune over a period of 6 months were included in the study. They were confirmed as *Acinetobacter* by the transformation assay (Brookes & Sodeman, 1972). Species identification of these isolates was done as per the biochemical typing scheme proposed by Bouvet & Grimont (1986).

Metal compounds

The metal salts were purchased from E. Merck (Darmstadt, Germany) as sodium meta arsenite, silver nitrate, cadmium chloride, cobalt nitrate, potassium chromate,

copper chloride, mercury chloride, sodium molybdate, nickel chloride, lead acetate, potassium antimony tartarate, sodium tungstate, aluminum chloride, bismuth sulfate, lithium chloride, selenium dioxide, strontium chloride, sodium arsenate, boric acid and zinc chloride. Stock solutions were made in distilled water and were sterilized by filtration through 0.22 μm membrane filters (Satorius). Freshly prepared solutions were used on the same day.

There are no standard metal concentrations to specify metal resistance (Trevors *et al.* 1985, Neito *et al.* 1989, Riley & Taylor, 1989). Therefore, the concentration range employed in previous studies, carried out with eubacteria, was chosen for determining the degree of resistance.

Susceptibility testing

The agar dilution method was used (Reeves *et al.* 1978) for the determination of the minimum inhibitory concentration (MIC) of heavy metals. Tubes containing GC agar supplemented with 5% yeast extract were used as described previously (Riley *et al.* 1989) and graded concentrations of metals were poured on plates. Plates were used on the same day, after drying at 37 °C for 1 h. Each plate was inoculated with 40 spots, each containing 10^4–10^5 bacteria from exponentially growing culture. The range of metal concentrations used was as follows (in mM ml^{-1}): 0.001, 0.01, 0.1, 1.0 and 10.0. Media without metals and inoculated with test organisms were used as controls. Plates were read after incubation at 37 °C for 2 days. The lowest concentration that completely inhibited growth was termed as MIC. Susceptibility assays for all metal compounds were repeated three times.

Detection of β-lactamase activity

β-Lactamase producing ability from intact cells was detected by the tube iodometric method (Catlin 1975). Those strains giving instant decolorization of the starch–iodine complex were regarded as β-lactamase-producer strains. The strains *Escherichia coli* K12 J53.2 and *E. coli* K12 J53.2 (RP4) were taken as negative and positive controls, respectively.

Results

Those isolates which gave positive transformation assays were confirmed as *Acinetobacter*. The strains were distributed in six different species (Table 1) by employing the biochemical tests. The β-lactamase-producer and -non-producer strains of *Acinetobacter* were distributed into different species as given in Table 1. *In toto*, 52% of strains showed production of β-lactamase as assayed from intact cells. β-Lactamase-producer and -non-producer strains were almost equally distributed in two of the species, i.e. *A. baumannii* and *A.* genospecies 1, whereas representatives of *A. lwoffii* genospecies 8/9, *Acinetobacter* genospecies 6 and *Acinetobacter* genospecies 10 lacked the ability to produce the enzyme. *A. baumannii* was a predominantly occurring and major β-lactamase producing species.

Table 1. Distribution of β-lactamase production in *Acinetobacter* species

Identified species	Number of strains	Number of β-lactamase producing strains
1. *A. baumannii*	31	18
2. *A.* genospecies 1	2	1
3. *A. junii* genospecies 5	1	1
4. *A. iwoffi* genospecies 8/9	2	1
5. *A.* genospecies 6	2	0
6. *A.* genospecies 10	1	0

Information based on responses of *Acinetobacter* isolates to the 20 metal ions emerged as belonging to three different categories (Tables 2–4). The first group (Table 2) consists of those metal ions to which almost all the *Acinetobacter* isolates are insensitive at upto 10 mM ml^{-1} concentration. Aluminum, bismuth and lithium showed no inhibitory effect on any of the strains tested. Boron inhibited one strain and molybdenum two strains at 10 mM ml^{-1} concentration. All three strains were β-lactamase negative.

Table 2. Resistance of *Acinetobacter* strains to metal ions (Group I)

Metal ion	β-Lactamase production	Number of strains having SIC[a] concentration (mM ml^{-1})				
		10[b]	1	0.1	0.01	0.001
Al	+	20	0	0	0	0
	−	19	0	0	0	0
Bi	+	22	0	0	0	0
	−	18	0	0	0	0
Li	+	20	0	0	0	0
	−	19	0	0	0	0
Bo	+	19	0	0	0	0
	−	18	1	0	0	0
Mo	+	19	0	0	0	0
	−	17	2	0	0	0
W	+	16	3	0	0	0
	−	16	3	0	0	0

[a]Subinhibitory concentration (the highest concentration tested at which bacteria showed growth).
[b]Strains counted under 10 mM ml^{-1} were capable of growing at that concentration. Higher concentrations were not tested.

Table 3. Resistance of *Acinetobacter* strains to metal ions (Group II)

Metal ion	β-Lactamase production	Number of strains having SIC concentration (mM ml^{-1})				
		10[a]	1	0.1	0.01	0.001
As^{3+}	+	16	2	1	0	0
	−	16	2	2	0	0
Cu	+	16	3	0	0	0
	−	13	7	0	0	0
Se	+	14	4	0	0	0
	−	11	8	2	0	0
Sn	+	13	7	0	0	0
	−	6	13	0	0	0
Pb	+	10	10	0	0	0
	−	4	15	0	0	0
As^{2+}	+	5	10	4	1	0
	−	2	13	4	0	0
Ag	+	6	7	5	0	0
	−	4	5	8	4	0

[a]Strains counted under 10 mM ml^{-1} were capable of growing at that concentration. Higher concentrations were not tested.

Table 4. Resistance of *Acinetobacter* strains to metal ions (Group III)

Metal ion	β-Lactamase production	Number of strains having SIC concentration (mM ml^{-1})				
		10[a]	1	0.1	0.01	0.001
Sb	+	0	7	12	2	0
	−	1	4	10	3	1
Cr	+	2	2	15	1	0
	−	0	2	12	5	0
Ni	+	0	14	6	0	0
	−	0	14	5	0	0
Co	+	0	0	20	0	0
	−	0	0	19	0	0
Zn	+	0	7	14	0	0
	−	0	6	12	0	0
Cd	+	0	1	8	8	2
	−	0	0	7	7	6
Hg	+	0	2	10	4	3
	−	0	1	7	3	9

[a]Strains counted under 10 mM ml^{-1} were capable of growing at that concentration. Higher concentrations were not tested.

The second group includes metal compounds which are more toxic to *Acinetobacter* strains at high concentrations (Table 3). All isolates except one β-lactamase producer belonging to *A. baumannii* and three non-producers (*A. baumannii*, *Acinetobacter* genospecies 1 and *Acinetobacter* genospecies 10) were sensitive to 0.1 mM ml^{-1} concentrations of arsenate and silver, respectively. At 10 mM ml^{-1} concentration a greater number of strains were β-lactamase producers. However, at 1 mM ml^{-1} more strains lacked the ability to produce β-lactamase, except for arsenite and silver. The third group of metals (Table 4) includes the more toxic metals. All strains, i.e. β-lactamase producers and non-producers, were uniformly sensitive to these metal ions at 10 mM ml^{-1}, except a few strains to antimony and chromium. In the case of chromium both the strains resistant at 10 mM ml^{-1} were β-lactamase producing *A. baumannii*. Resistance to nickel and zinc was equally distributed between β-lactamase producer and non-producer strains. All strains tested were sensitive to 10 and 1 mM ml^{-1} cobalt.

Cadmium and mercury appeared to be most toxic to *Acinetobacter* species. Resistance to these compounds was expressed only at low concentrations and was not related to the production of β-lactamase. Only one β-lactamase producer strain of *Acinetobacter* genospecies 1 was resistant to 1 mM ml^{-1} of cadmium ions. The next dominant resistant species to cadmium was *A. baumannii* (1 mM ml^{-1}). In the case of mercury, most resistant strains were β-lactamase producers and all those resistant to 1 mM ml^{-1} belonged to *A. baumannii*.

A. baumannii was thus highly metal resistant and a predominant β-lactamase producer. Other species of *Acinetobacter* contributed only a very small proportion of the sample size, making it difficult for us to draw any conclusions about their resistance to metal compounds in relation to β-lactamase production.

Discussion

Some metal ions are essential for the growth of microorganisms, while others are tolerated at extremely low concentrations. At high concentrations they prove to be toxic because they denature structural and functional proteins (Gadd & Griffiths 1978). Meers & Chow (1990) have reported that boric acid is weakly bactericidal to *Acinetobacter* at 1% (w/v) concentration and is only bacteriostatic at higher concentrations. Similarly, boron did not exert any inhibitory effect on *Acinetobacter* strains in this study at upto 10 mM concentration.

Acinetobacter is described as a natural producer of β-lactamase (Goldstein *et al.* 1983); however, in this study β-lactamase activity was detected from only 52% of isolates. The results suggest some kind of relationship between β-lactamase production and metal resistance in *Acinetobacter*. A larger proportion of metal resistant bacteria are β-lactamase producers, while the converse is not true. It has been postulated that β-lactamase protects the cell by non-specifically binding the antibiotic molecules, thus acting as a periplasmic 'sponge' which keeps the antibiotic from reaching the target site on the cytoplasmic membrane (Sanders 1983). Impermeability is a mechanism of resistance to both metals and antibiotics. The expolysaccharide produced by most of the *Acinetobacter* isolates may have a role in imparting this dual resistance.

The metal binding ability of *Acinetobacter* strains has been shown in a study on the removal of lead from solution. The metal binding ability of *A. calcoaceticus* was comparable with the ion exchange resin Amberlite IR-120 and occurred in the following sequence: Pb > Cu > Cr > Cd, Ni, Zn > Co (Mak *et al.* 1990). However, in the present study the degree of resistance to these metals occurred as: Cu > Pb > Cr > Ni > Co > Zn > Cd. Specific plasmid coded mechanisms have been identified for cadmium and mercury resistance (Chopra 1971, Tynecka *et al.* 1981, Silver & Misra 1984). These account for the sensitivity of *Acinetobacter* strains towards them. In this case β-lactamase genes may be on a plasmid associated with metal resistant as in the case of *Staphylococcus* (Novick & Roth 1968). Cadmium resistance is reported to be frequently associated with large penicillinase plasmids (Novick *et al.* 1979); however, in this study the cadmium resistant and cadmium sensitive strains were equally distributed between β-lactamase producers and non-producers. Antibiotic and metal resistance is shown to be present on the same plasmid in *Acinetobacter* (Dhakephalkar *et al.* 1992). The strains in this study also show multiple antibiotic resistance (data not shown). It is possible that many of these strains possess plasmids carrying metal and antibiotic resistance genes. This requires further investigation into the genetic nature of resistance. These studies are currently underway.

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