SUSCEPTIBILITY TO ANTIBIOTICS IN *ACINETOBACTER CALCOACETICUS*

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Acinetobacter calcoaceticus, a member of the family Neisseriaceae, belongs to the so-called nonfermentative bacteria. It is found as a ubiquitous organism in soil and water, and as a saprophyte on the skin and mucous membranes of human[1,2]. Although not an obligatory pathogen, the significance of *Acinetobacter* in human infection, especially in nosocomial infections, has been increasing over the last two decades[3,4].

We have reported[5~7], *A. calcoaceticus* (Glc$^-$) cannot grow in the presence of glucose as the sole source of carbon and Glc$^+$ is able to grow on glucose. We isolated 1,000 strains of *A. calcoaceticus* from patients, of which only 46 strains could grow on glucose. The strains that did not grow on glucose formed smooth and highly convex colonies on agar plates; colonies of the strains that grew on glucose were rough and flat. In addition we have isolated a mutant strain K-104G, that grows on glucose from strain K-104[5] which does not grow on glucose.

In this study, we examined the susceptibility of the various strains to various antibiotics and examined their β-lactamase activity.

Table 1 shows the *in vitro* antimicrobial activity of the test antibiotics against *A. calcoaceticus*. *A. calcoaceticus* NCTC 7844, K-104 and LMD 79.41 are Glc$^-$, *A. calcoaceticus* K-104G, KY 985 and LMD 82.3 are Glc$^+$. Gentamicin, kanamycin, tobramycin, polymyxin B and tetracycline had potent activity against both Glc$^-$ and Glc$^+$ strains. The MICs of benzylpenicillin and cefazolin for Glc$^-$ strains were higher than those for Glc$^+$ strains. In particular, the level of resistance to cefazolin was very high.

Among Glc$^-$ strains, the levels of resistance to β-lactam antibiotics were different. A characteristic difference in susceptibility between Glc$^-$ and Glc$^+$ strains was the level of resistance to chloramphenicol. The MICs of chloramphenicol for Glc$^-$ strains were 200 μg/ml or more, the Glc$^+$ strains were susceptible to chloramphenicol.

Six *A. calcoaceticus* strains were selected for further study. Crude β-lactamases prepared from these strains were examined for their substrate specificity and rates of hydrolysis by microiodometry. The rates of hydrolysis of benzylpenicillin and cefazolin are shown in Table 2. Only *A. calcoaceticus* NCTC 7844 showed high β-lactamase activity. The residual activity of

Table 1. Susceptibility to antibiotics of *Acinetobacter calcoaceticus*.

Antibiotics	MIC (μg/ml)[a]					
	NCTC 7844[b]	K-104	LMD 79.41	K-104G[c]	KY 985	LMD 82.3
Benzylpenicillin	1,600	50	200	3.13	6.25	12.5
Cefazolin	>3,200	400	1,600	50	100	100
Cefoperazone	800	50	100	6.25	12.5	12.5
Tetracycline	3.13	3.13	6.25	1.56	3.13	6.25
Minocycline	0.19	0.19	0.19	0.10	0.10	0.19
Gentamicin	0.78	0.78	1.56	0.39	0.19	0.78
Kanamycin	3.13	3.13	6.25	1.56	0.78	3.13
Tobramycin	1.56	1.56	3.13	0.78	0.78	3.13
Chloramphenicol	>400	200	>400	6.25	3.13	25
Polymyxin B	0.39	0.39	0.39	0.39	0.19	0.39

[a] Five μl of cell suspension (10^6 cells/ml) was inoculated onto sensitivity test agar containing the appropriate concentration of antibiotic.
[b] *A. calcoaceticus* NCTC 7844, K-104 and LMD 79.41 are Glc$^-$ strains.
[c] *A. calcoaceticus* K-104G, KY 985 and LMD 82.3 are Glc$^+$ strains.

benzylpenicillin and cefazolin was determined after incubating substrates with each strain. As we reported previously[8], *A. calcoaceticus* NCTC 7844 produced β-lactamase (cefalosporinase type) and hydrolyzed various β-lactam antibiotics. As shown in Table 3, *A. calcoaceticus* NCTC 7844 hydrolyzed benzylpenicillin and cefazolin rapidly, but the other strains did not hydrolyze either antibiotic even after 3 hours' incubation. We conclude that β-lactamase activity is not the main role in resistance of these strains to β-lactam antibiotics. Chloramphenicol acetyltransferase activity of Glc^+ and Glc^- strains was determined (the six strains shown in Table 1); chloramphenicol acetyltransferase was not detected in any strain (data not shown).

Table 2. β-Lactamase activity in *Acinetobacter calcoaceticus*.

Strain	Rate of hydrolysis (μmol/minute/mg protein)	
	Benzylpenicillin	Cefazolin
NCTC 7844	2.15×10^{-2}	7.04×10^{-2}
K-104	8.87×10^{-4}	3.87×10^{-4}
LMD 79.41	7.18×10^{-4}	1.73×10^{-3}
K-104G	2.59×10^{-4}	1.43×10^{-4}
KY 985	8.87×10^{-4}	9.29×10^{-4}
LMD 82.3	2.04×10^{-4}	2.36×10^{-4}

β-Lactamse assay was performed according to the method of OKONOGI *et al.*[11]. One-tenth ml of the substrate solution (final substrate concentration, 50 μg/ml) was added to 0.8 ml of phosphate buffer (pH 7.0) and incubated for 20 minutes at 30°C. Then the reaction was stopped with 0.15 ml of 0.15 M sodium tungustate and 1.5 ml of starch - iodine solution was added. After 20 minutes, the optical density at 595 nm was measured.

As reported previously[4~6], *A. calcoaceticus* lacks hexokinase and gluconokinase activities, and is therefore unable to assimilate glucose or gluconate. However it is able to oxidize several sugars to their lactones *via* glucose dehydrogenase. In this study, we investigated the susceptibility of two types of *A. calcoaceticus*, Glc^- and Glc^+, to antibiotics.

Glc^+ strains were susceptible to β-lactam and chloramphenicol antibiotics, whereas the Glc^- strains were resistant to these antibiotics. Except *A. calcoaceticus* NCTC 7844, neither Glc^- or Glc^+ strains had high β-lactamase activity nor showed chloramphenicol acetyltransferase activity. From these results, it could be concluded that β-lactamase does not play an important role in β-lactam antibiotic resistance, and that chloramphenicol acetyltransferase is not correlated with resistance to chloramphenicol.

In considering the mechanism of resistance to β-lactam antibiotics, it is interesting that β-lactam antibiotics are not effective against Glc^- strains. As we reported in another paper[5], Glc^- strains lack the 81K major outer membrane protein. Cephalosporin antibiotics are known to pass through the outer membrane mainly *via* porin pores[9]. The resistance of Glc^- strains to β-lactam antibiotics seems to be due their in-

Table 3. Residual activities of benzylpenicillin (PC-G) and cefazolin (CEZ).

Strain	Antibiotic	Residual activity (μg/ml)			
		0 hour	0.5 hour	1 hour	3 hours
NCTC 7844	PC-G	50	>0.78	>0.78	>0.78
	CEZ	50	>1.56	>1.56	>1.56
K-104	PC-G	50	50	50	33.2
	CEZ	50	41.6	34.5	36.0
LMD 79.41	PC-G	50	50	50	50
	CEZ	50	38	35.2	32.7
K-104G	PC-G	50	50	50	50
	CEZ	50	42	43.6	38.5
KY 985	PC-G	50	50	50	39.2
	CEZ	50	50	47.2	40.7
LMD 82.3	PC-G	50	50	50	50
	CEZ	50	44	48	42.5

Residual activities were assayed by *Bacillus subtilis* ATCC 6633 as a test organism.

ability to permeate the outer membrane. In the case of chloramphenicol, the resistance also seems to be due to its inability to permeate the membrane of Glc^- strains.

Among the antibiotics tested, tetracycline and the aminosugar antibiotic kanamycin were very effective against both Glc^+ and Glc^- strains. These antibiotics are known to be carried into cells by active transport systems. As we reported previously[5~7], *A. calcoaceticus* has a specific energy system which is due to membrane-bound glucose dehydrogenase which transfers electrons from glucose to the electron transport system. *A. calcoaceticus* is able to generate a proton gradient and ATP in the presence of glucose by coupling with this system, and the oxidation of glucose stimulates the active transport of various substrates into cells.

In addition, there were some aminosugar-resistant (gentamicin- or amikacin-resistant) strains among both Glc^+ and Glc^- strains. These strains produce various aminosugar-inactivating enzymes. Several years ago, we investigated aminosugar-inactivating enzymes and reported that *Serratia marcescens*, *Serratia* sp., *Serratia proteamaculans*, all clinical isolates, produced a new type of aminoglycoside acetyltransferase which acetylated amikacin at the 6′-amino group[10]. In *A. calcoaceticus*, various aminoglycoside-inactivating enzymes were found, as will be reported elsewhere.

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