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Susceptibility of *Mycobacterium bovis* BCG Vaccine Strains to Antituberculous Antibiotics[▽]

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***Mycobacterium bovis* BCG is one of the most commonly administered vaccines. Complications, including disseminated BCG disease, are rare but increasingly reported in immunodeficient children. There is growing recognition of the importance of differences between BCG vaccine strains. We determined the susceptibilities of five genetically distinct BCG vaccine strains to 12 antituberculous drugs.**

Mycobacterium bovis bacillus Calmette-Guérin (BCG) vaccine is one of the most commonly administered vaccines worldwide. It is a live attenuated vaccine derived from *M. bovis*, and for historical reasons a number of genetically different BCG vaccine strains are in use today (3, 17). In immunocompetent individuals local adverse reactions such as injection site abscess and lymphadenitis occur in less than 1% of vaccinees (21) and disseminated BCG disease is extremely rare (12). However, in individuals with congenital or acquired immunodeficiency the risk of local complications and disseminated BCG disease after immunization is considerably greater (7). Recent reports from regions with a high prevalence of human immunodeficiency virus suggest that BCG-related complications are becoming increasingly common (7). The appropriate treatment of local adverse reactions and disseminated BCG disease remains controversial, and the influence of the particular BCG vaccine strain or its susceptibility pattern is not considered (6, 8, 9). There are only limited data on the susceptibility of BCG to different antituberculous drugs and even less on the influence of genetic differences between BCG vaccine strains on susceptibility.

BCG vaccine strains were kindly provided by their manufacturers as follows: BCG-Bulgaria (SL 222 Sofia), BB-NCIPD Ltd., Sofia, Bulgaria; BCG-Denmark (SSI 1331), Statens Serum Institute, Copenhagen, Denmark; and BCG-Japan (Tokyo 172) Japan BCG Laboratory, Tokyo, Japan. BCG-Connaught (Sanofi Pasteur, Toronto, Canada) and BCG-Medac (RIVM from 1173-P2; Medac, Hamburg, Germany) were purchased. Freeze-dried BCG vaccine strains were dissolved in 0.5 ml of 0.9% saline (10 ml for BCG-Medac) and cultured using the MB/BacT system (Biomérieux, Durham, NC) according to manufacturer's instructions and subsequently subcultured on solid media (Lowenstein-Jensen and Brown & Buckle agar) for antimicrobial susceptibility testing. Susceptibility testing was done using the Bactec MGIT (mycobacterial growth indicator tube) 960 system (Becton Dickinson, MD) according to the manufacturer's instructions. Lyophilized streptomycin, isoniazid, rifampin (rifampicin), and ethambutol (Bactec MGIT SIRE kit; Becton Dickinson, MD) were each reconstituted and used according to the manufacturer's instructions. Amikacin, capreomycin, ethionamide, kanamycin, and ofloxacin (all Sigma), ciprofloxacin (Bayer), and rifabutin (Farm Italia, Carlo Erba, Research Laboratories) were dissolved and used at the recommended concentrations (Table 1) (11). For susceptibility testing of all drugs except pyrazinamide, the drug-containing tubes were inoculated with a suspension prepared from the culture on solid media and adjusted to a 0.5 McFarland standard in turbidity. The tubes were incubated and monitored for growth until the results indicating susceptibility or resistance were automatically interpreted by the BACTEC MGIT 960 instrument. Susceptibility to pyrazinamide was tested using Wayne's indirect method, measuring the presence of pyrazinamidase (22).

The individual BCG vaccine strains were flagged positive after the following durations of culture: 2.5 days, BCG-Medac; 2.7 days, BCG-Japan; 4.0 days, BCG-Connaught; 5.2 days, BCG-Denmark; and 6.2 days, BCG Bulgaria. Results for the testing of susceptibilities to different antituberculous drugs are shown in Table 1.

This is the first study to compare the susceptibilities of the most commonly used BCG vaccine strains worldwide to a wide range of antituberculous drugs. Previous studies in this area have been limited by the use of a single BCG strain (5) or alternatively by the limited range of antituberculous drugs tested (summarized in Table 2).

Broth-based susceptibility testing using nonradiometric systems, such as the Bactec MGIT 960 system, are now widely used for susceptibility testing of mycobacteria, and the correlation between in vitro testing at a critical antibiotic concentration and clinical outcome is well documented for *Mycobacterium tuberculosis* (18). Although the correlation between in vitro susceptibility results and clinical outcome has not been studied for BCG disease, susceptibility results obtained by this

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TABLE 1. Susceptibilities of different BCG vaccine strains to antituberculous drugs

Antituberculous drug	Concn (μg/ml)	Susceptibility[a] of BCG strain:				
		BCG-Bulgaria (SL 222 Sofia)	BCG-Connaught	BCG-Denmark (SSI 1331)	BCG-Japan (Tokyo 172)	BCG-Medac (RIVM from 1173-P2)
First-line antituberculous drugs						
Isoniazid	0.1	S	R	R	S	S
Isoniazid	0.4	S	S	S	S	S
Rifampin	1.0	S	S	S	S	S
Rifabutin	0.5	S	S	S	S	S
Pyrazinamide	100.0	R	R	R	R	R
Ethambutol	5.0	S	S	S	S	S
Second-line antituberculous drugs						
Ethionamide	5.0	S	R	R	S	S
Ciprofloxacin	1.0	S	S	S	S	S
Ofloxacin	1.0	S	S	S	S	S
Streptomycin	1.0	S	S	S	S	S
Amikacin	1.0	S	S	S	S	S
Kanamycin	5.0	S	S	S	S	S
Capreomycin	2.5	S	S	S	S	S

[a] R, resistant; S, sensitive.

method are considered clinically relevant as *M. tuberculosis* and BCG both belong to the *M. tuberculosis* complex.

Three of the five tested BCG vaccine strains (BCG-Bulgaria, BCG-Japan, and BCG-Medac) were susceptible to all antituberculous drugs except pyrazinamide, to which *M. bovis* and consequently all BCG vaccine strains are intrinsically resistant.

BCG-Connaught and BCG-Denmark both showed low-level resistance to isoniazid. Resistance of BCG-Denmark to isoniazid is documented by the vaccine manufacturer (Statens Serum Institute; www.ssi.dk/sw10375.asp, 1993), but the clinical significance of this resistance is unclear. The Global Advisory Committee of Vaccine Safety of the World Health Organization reported five patients with isoniazid-resistant BCG lymphadenitis after immunization with BCG-Denmark but concluded that the identification of low-level isoniazid resistance does not justify any change in BCG immunization policy (23). In addition, serum concentrations in children after a dose of 10 mg/kg of body weight isoniazid significantly exceed 0.4 μg/ml (19), suggesting that low-level resistance may not be clinically significant.

Resistance of BCG-Connaught and BCG-Denmark to ethionamide has not previously been documented. The association between resistance to isoniazid and ethionamide is found in other mycobacteria. It was first described for isolates of *M.*

TABLE 2. Susceptibilities of different BCG vaccine strains to antituberculous drugs using different methods of susceptibility testing

Antituberculous drug	MIC (mg/liter) for BCG vaccine strain[a]:						
	BCG-Russia (16)	BCG-Connaught (5)	BCG-Denmark (SSI 1331) (9, 14–16[b])	BCG-Japan (Tokyo 172) (14, 20)	BCG-Moreau (4)	BCG-Pasteur (4, 14, 16)	BCG-Glaxo (14, 15)
First-line antituberculous drugs							
Isoniazid		0.125	0.4	0.06		0.1	
Rifampin		<0.031	0.016–2	0.063–0.5		0.063–0.125	0.031
Rifabutin		0.063	0.016	0.063		0.063–0.125	0.016
Pyrazinamide		>800					
Ethambutol		2	2.5	2–4			2.5
Second-line antituberculous drugs							
Ethionamide		4					
Ciprofloxacin		0.125					
Ofloxacin	1	<0.031	0.75			0.75	
Streptomycin		0.25	2	0.25			2
Amikacin		0.125					
Kanamycin		1					
Capreomycin		0.5					
Macrolides							
Azithromycin		16				1	
Clarithromycin		0.25	0.07–0.15		2	0.3–0.6	0.15–0.3
Erythromycin		16			128	4	

[a] Alternate strain names and references are in parentheses.
[b] See also the summary of BCG product characteristics at www.ssi.dk/sw10375.asp.

tuberculosis from patients who had been on isoniazid monotherapy, suggesting that isoniazid and ethionamide share a common target (10). Subsequently, the gene *inhA*, whose mutation or overexpression leads to resistance against isoniazid and ethionamide, was identified in *Mycobacterium smegmatis* (2). A recent study found that all *M. tuberculosis* isolates with low-level isoniazid resistance that were also ethionamide resistant had mutations in the regulatory region of the *inhA* gene (1). In contrast, only 5.9% of isolates with high-level isoniazid resistance that were also ethionamide resistant had such mutations.

In some countries ethionamide is the preferred drug to be included in combination treatment of tuberculous meningitis and is used in cases of disseminated BCG disease with presumed central nervous system involvement. However, if the vaccine strain used for immunization was BCG-Connaught or BCG-Denmark, then our data suggest that an alternative treatment strategy should be used.

We were unable to test susceptibility to erythromycin, as clinically meaningful concentrations of macrolides for in vitro susceptibility testing are not available for *M. tuberculosis* or *M. bovis*. Treatment with erythromycin is controversial but is frequently recommended for localized complications after BCG immunization despite limited evidence of efficacy (6). However, in vitro evidence suggests that BCG vaccine strains that have lost region of deletion 2 (RD2) are susceptible to macrolides, whereas those which have retained RD2 (such as BCG-Japan, BCG-Russia, and BCG-Moreau) are resistant (4).

There is growing concern about the rising incidence of BCG-related complications associated with the human immunodeficiency virus/AIDS epidemic (7, 9) and the lack of evidence-based guidelines for the treatment of local and disseminated complications (13). Our study indicates that BCG vaccine strains differ in their susceptibilities to antituberculous drugs and suggests that these differences should be taken into account when selecting empirical treatment for BCG-related complications. This study also emphasizes the importance of genetic differences between BCG vaccine strains and highlights the need to document the particular BCG vaccine strains used in different countries (17). As different strains are sometimes used in the same country, recording the strain administered in an individual infant's immunization record would also be helpful.

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