

## Transferable Chloramphenicol and Ampicillin Resistance in a Strain of *Haemophilus influenzae*

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A strain of *Haemophilus influenzae* isolated from sputum has been shown to be resistant to chloramphenicol, ampicillin, and tetracycline. All three resistances were transferred together to recipients when the selecting antibiotic was ampicillin, chloramphenicol, or tetracycline.

Strains of *Haemophilus influenzae* resistant to ampicillin have been frequently reported over the past several years (2, 5-8, 10, 13). Occasional strains resistant to chloramphenicol and other antibiotics have also been detected (3, 4, 9, 12). At the time of this writing, however, no strain resistant to both chloramphenicol and ampicillin has been reported. The presence of such resistance is of major clinical consequence since these are the drugs of choice in meningitis due to *H. influenzae*.

Recently we isolated a strain of *H. influenzae* from a sputum sample of a 9-year-old boy at the University of Alberta Hospital in Edmonton. The strain, designated *H. influenzae* UAHf-1, was confirmed to be *H. influenzae* by standard procedures (14). The organism was not associated with an apparent infection and was nontypable. It was determined to be resistant to ampicillin and chloramphenicol by both disk diffusion and tube dilution testing and to tetracycline by disk diffusion testing (Table 1). Antimicrobial susceptibility testing was carried out with Mueller-Hinton medium supplemented with 5% Fildes digest of blood (MHF). The inoculum was prepared by growth in MHF broth to a density of about  $10^8$  cells per ml. In broth dilution tests the final inoculum was  $10^4$  cells per ml, and the result was read after 24 h of incubation in 5% CO<sub>2</sub> at 37°C. Disk diffusion testing was performed by inoculating MHF agar as described for the standard Bauer et al. procedure (1), and plates were incubated as described.  $\beta$ -Lactamase testing was carried out by using an acidometric test previously described (11). The minimum inhibitory concentrations of chloramphenicol and ampicillin were several times that for a standard susceptible strain tested under the same conditions. Strain UAHf-1 also had  $\beta$ -lactamase activity.

Strain UAHf-1 was examined for transfer of chloramphenicol or ampicillin resistance to a recipient strain, *H. influenzae* UAHf-2 Rif<sup>r</sup>. The

latter strain is a derivative of a clinical isolate which is resistant to 100  $\mu$ g of rifampin per ml. Strains for transfer experiments were grown in MHF broth to an absorbance at 600 nm of 0.2. Equal volumes of the donor and recipient were mixed, and a 5-ml volume was filtered through a membrane filter (pore size, 0.22  $\mu$ m; Millipore Corp.). This filter was placed on MHF agar and incubated in 5% CO<sub>2</sub> at 37°C. Samples were removed at 2 and 24 h with a bacteriological loop and resuspended in MHF broth to  $10^9$  cells per ml. A 0.1-ml volume of resuspended cells was plated on MHF agar with rifampin (100  $\mu$ g/ml) and either ampicillin (1  $\mu$ g/ml) or chloramphenicol (8  $\mu$ g/ml) and incubated at 37°C for 24 h in 5% CO<sub>2</sub>. The results of this mixed culture are given in Table 2. This procedure was repeated with colonies selected as probable transconjugants from the above mixed culture used as donors and a derivative of *H. influenzae* UAHf-2 resistant to 1,000  $\mu$ g of streptomycin per ml, using streptomycin (1,000  $\mu$ g/ml) for counterselection. Resistance to ampicillin (and  $\beta$ -lactamase activity), chloramphenicol, and tetracycline was transferred twice (Tables 1 and 2). This protocol confirms that the resistances were transferable particularly in the second mixed culture. Under the latter circumstances a double mutation (streptomycin resistance reverting to susceptibility and rifampin susceptibility mutating to resistance) in the recipient is necessary to explain the results otherwise. It has also been possible to transfer combined chloramphenicol, ampicillin, and tetracycline resistance by selection on chocolate agar plates with 8  $\mu$ g of tetracycline per ml and 100  $\mu$ g of either rifampin or streptomycin per ml. Thus, it appears that the three resistances are linked or, if on separate plasmids, there is a high incidence of coincidental transfer of the plasmids. We have not yet examined these strains for physical evidence of a plasmid(s).

The presence of transferable combined ampi-

TABLE 1. Antimicrobial susceptibility of various strains of *H. influenzae*

<i>H. influenzae</i> strain	Source and characteristics	$\beta$ -Lactamase	Disk susceptibility (zone diam, mm)										Minimum inhibitory concn ( $\mu$ g/ml)			
			P <sub>10</sub> <sup>a</sup>	A <sub>10</sub>	C <sub>5</sub>	C <sub>30</sub>	T <sub>30</sub>	E <sub>15</sub>	Gm <sub>10</sub>	Sep <sub>25</sub>	R <sub>50</sub>	Str <sub>25</sub>		Ampicillin	Chloramphenicol	
UAHf-1	Clinical isolate Laboratory strain, recipient 1; "standard sensitive strain"	+	R <sup>b</sup>	R	9	15	15	15	15	20	22	34	25	15	4	16
UAHf-2 Rif <sup>r</sup>		-	20	25	24	40	30	30	30	19	20	38	R	15	0.125	1
UAHf-2 Rif <sup>r</sup> transconjugant	Transconjugant from 2-h mating (UAHf-1 x UAHf-2 Rif <sup>r</sup> )	+	R <sup>c</sup>	R	9	15	11	11	18	20	39	R	15	4	16	
UAHf-2 Str <sup>r</sup>	Streptomycin-resistant derivative of the parent of strain UAHf-2 Rif <sup>r</sup>	-	20	25	24	40	30	30	19	20	36	22	R	0.125	1	
UAHf-2 Str <sup>r</sup> transconjugant	Transconjugant from 2-h mating (UAHf-2 Rif <sup>r</sup> transconjugant x UAHf-2 Str <sup>r</sup> )	+	R	10	10	14	11	11	20	22	37	25	R	4	16	

<sup>a</sup> Type of disk and antibiotic content per disk: P<sub>10</sub>, penicillin, 10  $\mu$ g; A<sub>10</sub>, ampicillin, 10  $\mu$ g; C<sub>5</sub>, chloramphenicol, 5  $\mu$ g; C<sub>30</sub>, chloramphenicol, 30  $\mu$ g; T<sub>30</sub>, tetracycline, 30  $\mu$ g; E<sub>15</sub>, erythromycin, 15  $\mu$ g; Gm<sub>10</sub>, gentamicin, 10  $\mu$ g; Sep<sub>25</sub>, septr, 25  $\mu$ g; R<sub>50</sub>, rifampin, 50  $\mu$ g; Str<sub>25</sub>, streptomycin, 25  $\mu$ g.

<sup>b</sup> R, No zone.

<sup>c</sup> Disk susceptibilities underlined are those changing in the recipient after mating.

TABLE 2. *Transfer of antibiotic resistance from H. influenzae UAHf-1<sup>a</sup>*

Donor	Recipient	No. of colonies when selected on:		No. of chloramphenicol-resistant colonies resistant to ampicillin and tetracycline
		Ampicillin <sup>b</sup>	Chloramphenicol <sup>b</sup>	
Mating series 1				
UAHf-1	UAHf-2 Rif <sup>r</sup>	0	33	10/10
UAHf-1	None (control)	0	0	
None	UAHf-2 Rif <sup>r</sup> (control)	0	0	
Mating series 2				
UAHf-2 Rif <sup>r</sup> Str <sup>s</sup> <sup>c</sup>				
Transconjugant	UAHf-2 Str <sup>r</sup> Rif <sup>s</sup> <sup>c</sup>	ca. 800	ca. 800	10/10
UAHf-2 Rif <sup>r</sup> Str <sup>s</sup>				
Transconjugant	None (control)	0	0	
None	UAHf-2 Str <sup>r</sup> Rif <sup>s</sup> (control)	0	0	

<sup>a</sup> Filter mating for 24 h.

<sup>b</sup> Contransselection in mating 1 was with rifampin, 100 µg/ml, and in mating 2 with streptomycin, 1,000 µg/ml. The total number of cells spread on selective plates was about 10<sup>8</sup>.

<sup>c</sup> Rif<sup>r</sup>, Resistant to 100 µg of rifampin per ml; Rif<sup>s</sup>, susceptible to 100 µg of rifampin per ml; Str<sup>r</sup>, resistant to 1,000 µg of streptomycin per ml; Str<sup>s</sup>, susceptible to 1,000 µg of streptomycin per ml.

cillin and chloramphenicol resistance in *H. influenzae* adds another serious aspect to the therapy of childhood bacterial meningitis. Although such combined resistance is obviously still rarely detected, a similar situation also once existed for ampicillin resistance. The physician can no longer be as confident that combined chloramphenicol and ampicillin treatment will be effective for all strains of *H. influenzae*. In addition to the strain noted above, we have detected about 5 to 7% of all clinical isolates of *H. influenzae* as resistant to ampicillin. Occasional strains resistant to chloramphenicol and tetracycline but susceptible to ampicillin have also been found. Transferable resistance has been detected in representative strains showing both of these resistance patterns.

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