Resistance of Haemophilus influenzae to Trimethoprim

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Summary
Out of 210 isolates of Haemophilus influenzae obtained from the sputum of 63 patients with chronic respiratory infections 109 (52%) were resistant to trimethoprim-sulphamethoxazole by the disc test. The minimal inhibitory concentrations of trimethoprim for 17 out of 18 strains recorded as resistant were 10 μg/ml or higher. Resistant strains were isolated from time to time from 32 (82%) of 39 patients known to have been treated with trimethoprim-sulphamethoxazole, compared with only 1 (12.5%) out of 8 patients known not to have been treated with this drug combination. Resistant strains were isolated most frequently from patients who had received long-term treatment. Since sulphamethoxazole penetrates from the blood into the bronchial secretions less readily than does trimethoprim it seems likely that the ratio of the two drugs in the bronchial tree is far from ideal. This may be an important factor in the use of these drugs for chest infections.

Introduction
The minimal inhibitory concentration (M.I.C.) of trimethoprim for Haemophilus influenzae is usually about 0.1-1 μg/ml (Bushby, 1969), but Waterworth (1969) reported the isolation in our laboratory of two strains for which the M.I.C.s were 32 and 16 μg/ml respectively. Commenting on this observation, Garrod and O'Grady (1971) remarked: "Such emergence of resistance so relatively early in the drug's therapeutic history must again be taken as a warning that it should not be used for infections which are almost certainly not of bacterial origin, for trivial infections which do not require antibacterial therapy, or for infections which can equally successfully and conveniently be treated with something else." Our experience of the occurrence of trimethoprim-resistant H. influenzae during the past four years strongly reinforces the need for this warning. We report here the details of this experience.

Material and Methods
The survey is based on the findings of routine sensitivity tests of 210 isolates of H. influenzae cultured from the sputum of 63 patients investigated between June 1968 and February 1972. Forty-five patients were suffering from cystic fibrosis and 18 had other chronic respiratory disorders—namely, chronic bronchitis, asthma, bronchiectasis, or bronchial carcinoma.

Sensitivity Tests.—It is well known that tests of sensitivity of micro-organisms to trimethoprim and sulphonamides can give erroneous results if the medium used for the tests contains large amounts of the end-products of folate metabolism (Bushby, 1969). These "inhibitors" enable the organism to escape the blockade of folate synthesis and thus to grow in the presence of the drugs. In consequence, organisms may appear to be drug-resistant when they are, in fact, sensitive. When confronted with an unexpectedly high rate of resistance to these drugs, therefore, it is necessary to scrutinize the possibility of experimental error of this origin. Accordingly we describe below, firstly, our routine test procedure and, secondly, tests carried out to confirm or refute the findings.

Routine Sensitivity Test Procedure.—Routine sensitivity determinations were carried out by the filter-paper disc method, using standard discs (Oxoid) containing 25 μg of a mixture of trimethoprim (1 part) and sulphamethoxazole (19 parts). Nutrient agar plates (see below) were flooded with six-hour broth cultures of the test strains diluted 1:100. The excess was pipetted off and the plates were dried at 37°C, the discs applied, and the plates incubated at 37°C overnight. Three types of result were observed: (1) complete inhibition of growth around the disc; (2) an "inhibition zone," but containing minute colonies throughout; and (3) normal growth around the disc. A strain was recorded as being drug-resistant if the zone of complete inhibition extended for less than 2 mm from the edge of the disc. The culture medium used for these tests was based on the meat-digest broth devised by Breach and his colleagues at Westminster Hospital (Baker and Breach, 1967). To this were added X factor (haemin, B.D.H.) and V factor (nicotinamide adenine dinucleotide, B.D.H.) to a final concentration of 3 and 0.3 mg/100 ml w/v respectively. Lysed horse blood, at a final concentration of 0.5%, was also added in order to clear the medium of inhibitors of trimethoprim and sulphonamides (Bushby, 1969; Waterworth, 1969).

Confirmation Tests.—Twenty-four strains were available for repeat tests. These took the form of estimations of the M.I.C.s of trimethoprim on media known to be free from inhibitors—namely, Diagnostic Sensitivity Test Agar (D.S.T.A., Oxoid).
and Wellcome Nutrient Agar—to each of which 5", lyed horse blood was added together with X and V growth factors. Plates were poured of these media containing serial dilutions of tri-
methoprim ranging from 20 μg to 0.6 μg ml, and three-hour 
broth cultures of the organisms were streaked on each. After 
night incubation at 37 C the M.I.C. for each organism was 
noted as the lowest concentration of trimethoprim in the 
presence of which no growth could be seen.

Results

Routine Tests.—Of the 210 H. influenzae isolates tested 109 (52\(^\circ\).) were found to be resistant to the trimethoprim-sul-
phamethoxazole disc. Most of the 63 patients from whom the 
isolates were obtained had undergone repeat examinations over 
many months. Resistant strains were isolated at one time or 
another from 40 patients (63\(^\circ\).). In many instances sensitive 
strains were also found from time to time, suggesting that the 
patients were often infected with more than one strain of H. 
influenzae. It should be noted that many of the strains recorded 
as resistant showed, at first glance, inhibition zones around the 
disc, but closer inspection revealed minute colonies growing 
throughout the zone. This phenomenon is discussed further 
below.

M.I.C.s.—M.I.C.s of trimethoprim were determined for 24 
strains on D.S.T.A. + 5", lyed blood. In the routine test 18 
of these had been recorded as “resistant,” and their M.I.C.s 
were: 14 strains > 20 μg/ml, 3 strains 10 μg/ml, and 1 strain 
1.2 μg/ml. Of six strains recorded as “sensitive” the M.I.C.s 
were for five 0.6-2.5 μg/ml, while that for the remaining one was 
> 20 μg/ml. The latter was the only strain showing a gross 
crepancy between the two types of test. M.I.C.s for four strains 
were determined on Wellcome Nutrient Agar + 5", lyed blood 
and compared with those on D.S.T.A. In each instance the 
M.I.C. on both media was >20 μg/ml. As with the disc tests the 
growth of resistant strains in the presence of the higher 
concentrations of trimethoprim usually consisted of minute 
colonies, and the end-points of titrations were seldom clear-cut.
This appearance was also noted by Miss Pamela Waterworth, 
whom the strains were sent for independent examination.

Occurrence of Resistant Strains in Relation to Treatment.—
Thirty-nine patients were known to have been treated with 
trimethoprim-sulphamethoxazole. Resistant H. influenzae was 
isolated from time to time from 32 (82\(^\circ\).) of them. In contrast

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>Treatment with T-S</th>
<th>No. of Patients From Whom H. influenzae was Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>Yes</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>8</td>
</tr>
<tr>
<td>Patients with cystic fibrosis</td>
<td>Usually long-term</td>
<td>32</td>
</tr>
<tr>
<td>Patients with other disorders</td>
<td>Usually short-term</td>
<td>17</td>
</tr>
</tbody>
</table>

only 1 (12.5\(^\circ\).) resistant strain was found among the strains 
isolated from each of 8 patients who, so far as was known, had 
not taken trimethoprim-sulphamethoxazole. It was not possible 
to analyse precisely the occurrence of resistant strains in rela-
tion to duration of treatment, since regimens varied enormously.

In general, however, it seems justified to distinguish between 
the patients with cystic fibrosis, who were often given long 
courses of treatment, and those with other diseases, whose 
treatment was usually shorter. Thus H. influenzae was isolated 
on various occasions from 32 patients with cystic fibrosis known 
to have been treated with trimethoprim-sulphamethoxazole, 
28 (87.5\(^\circ\).) yielding resistant strains from time to time. In 
comparison 4 (57\(^\circ\).) of the strains isolated from each of 7 
patients with other disorders treated with trimethoprim-sulphamethox-
azole were resistant to the mixture. These findings are summarized in the Table.

Discussion

The occurrence of small colonies within the inhibition zone in a 
disc test for sensitivity to trimethoprim is suggestive of inade-
quately clear concentrations in the blood. For most bacterial species this ratio is about 1 part trimetho-
prim to 20 parts sulphamamide. Commercially available ther-
apeutic preparations (Septrin, Bactrom) contain one part tri-
methoprim to five parts sulphamethoxazole, which, after 
differential absorption from the bowel, give a 1 : 20 ratio of con-
centrations in the blood. Unfortunately, however, sulphamethoxazole penetrates from the blood into bronchial secretions 
less readily than does trimethoprim, and the resulting ratio 
there is of the order of 1 : 1 (Reeves, 1971). As a result not only 
would the antibacterial activity of the drugs be expected to be 
suboptimal in respiratory infections but the value of the sul-
phamamide in reducing the risk of emergence of trimethoprim-resistant strains would be largely lost.

It is possible that this defect might be remedied by greatly 
raising the dose of sulphamethoxazole for the treatment of respiratory infections, although it is probable that considera-
tions of toxicity would preclude an increase sufficient to give a 
20-fold increase in the concentration attainable in bronchial secretions. Even so, there is clearly a case for the use of dose ratios of trimethoprim and sulphamamide more closely related to the needs of the site of the infection being treated; and official 
recognition of the acceptability of a single mixture, under the 
name "co-trimoxazole," to be used for infections in any situa-
tion, is regrettable.

We wish to thank Miss Pamela Waterworth for her advice and 
for examining some of our strains, and Mrs. C. R. Laughton 
for technical help.

References