IN-VITRO SENSITIVITY OF SHIGELLA SONNEI TO TRIMETHOPRIM AND SULPHAMETHOXAZOLE

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The value of a combination of trimethoprim and sulphamethoxazole in the treatment of a variety of infections is now well established. Little has yet been published, however, on the possible effectiveness of the combination in bacillary dysentery. As a first step it was thought that it would be of interest to ascertain the in-vitro sensitivity of recently isolated strains of Shigella sonnei to the two drugs. A collection of 209 strains of Sh. sonnei of various colicine types and antibiotic-resistance patterns were tested; these were isolated mainly in the Greater London Area.

MATERIALS AND METHODS
Most of the strains of Sh. sonnei tested were isolated from specimens submitted to the Public Health Laboratory, St George’s Hospital, London, and the Public Health Laboratory,

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County Hall, London, in the first 6 mth of 1969. A few had been referred for colicine typing from the Public Health Laboratory, Maidstone, Kent. The strains were therefore current in the London Boroughs of Bexley, Camden, Hackney, Hammersmith, Islington, Kensington and Chelsea, Lambeth, Merton, Southwark, Wandsworth, the City of Westminster, and the County of Kent.

The methods used for isolation, for identification, and for determination of antibiotic-resistance pattern of the strains of Sh. sonnei were those of Farrant and Tomlinson (1966); colicine typing was also performed (Abbott and Shannon, 1958; Abbott and Graham, 1961). Strains were selected on the basis of one isolate per incident, an incident being defined as one or more cases in a family or day nursery. Strains were stored on nutrient agar slopes, and their purity and identity were checked immediately before testing by plating out and by slide agglutination.

### Table I

Minimum inhibitory concentration (MIC) of trimethoprim and sulphamethoxazole for 209 strains of Shigella sonnei

<table>
<thead>
<tr>
<th>Number (and percentage) of strains for which the MIC (µg per ml) of</th>
<th>trimethoprim was</th>
<th>sulphamethoxazole was</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>1 (&lt;1)</td>
<td>163 (78)</td>
</tr>
<tr>
<td></td>
<td>45 (22)</td>
<td>13 (6)</td>
</tr>
<tr>
<td></td>
<td>40 (19)</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td></td>
<td>155 (74)</td>
<td></td>
</tr>
</tbody>
</table>

Minimum inhibitory concentrations (MICs) were determined by a plate dilution method. The medium used was Diagnostic Sensitivity Test Agar (Oxoid) containing 5 per cent. horse blood lysed by saponin. The drugs were incorporated into the medium to give two-fold differences between adjacent plates in the series. The plates used for demonstrating potentiation contained trimethoprim and sulphamethoxazole in a ratio of 1:20 respectively by weight; in this series also, there was a two-fold difference between adjacent plates.

The organisms for MIC determination were grown overnight in nutrient broth and then diluted 1000-fold in sterile distilled water. A 3-mm loopful of the diluted culture was spread over a circular area of agar approximately 1 cm in diameter; the dose of organisms resulting from this was found to be about 300. Twenty strains of Sh. sonnei were tested on each plate. The tests were made in two batches, but those against a particular strain, with each drug separately and with the combination, were performed together on medium of the same batch and with the same series of antibiotic dilutions.

### Results

All strains of Sh. sonnei tested were sensitive to trimethoprim at a concentration of 0.32 µg per ml; but only 26 per cent. were sensitive to sulphamethoxazole at a concentration of 6.4 µg per ml, the remainder being resistant at a concentration of 100 µg per ml (table I). Table II shows the results of testing for potentiation; these are recorded as the reduction in MIC of trimethoprim brought about by the presence of sulphamethoxazole. Well-marked potentiation of the trimethoprim was demonstrated in the case of the sulphonamide-sensitive strains, but there appeared to be little or no potentiation against sulphonamide-resistant strains. Ninety-two per cent. of strains were resistant to ampicillin, 61 per cent. to streptomycin, and 27 per cent. to tetracycline; occasional strains were resistant to neomycin, kanamycin, and chloramphenicol. The strains were of the following colicine types: 0, 2, 3, 3a, 4, 6, 7, 8, and 9.
DISCUSSION

All strains of *Sh. sonnei* tested were very sensitive to trimethoprim, but 74 per cent. were resistant to sulphamethoxazole. The proportion of resistant strains has fallen compared with previous surveys (Scrimgeour, 1966; Davies, Farrant and Tomlinson, 1968) that covered the same geographical area.

It was possible to demonstrate potentiation of the trimethoprim by sulphamethoxazole against sulphonamide-sensitive strains, but not against sulphonamide-resistant strains. The ratio of the two drugs used in the tests for potentiation was about the same as the ratio of their MICs for sulphonamide-sensitive strains, that is to say 20 parts by weight of sulphonamide to one part of trimethoprim. This mixture was therefore probably not optimal for demonstrating potentiation against sulphonamide-resistant strains, but tests with a mixture containing much more sulphonamide would not have been comparable with the therapeutic situation.

<table>
<thead>
<tr>
<th>Potentiation of trimethoprim*</th>
<th>Number of strains showing the stated amount of potentiation among</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sulphonamide-sensitive strains</td>
</tr>
<tr>
<td>× 0.5</td>
<td>0</td>
</tr>
<tr>
<td>× 1</td>
<td>0</td>
</tr>
<tr>
<td>× 2</td>
<td>0</td>
</tr>
<tr>
<td>× 4</td>
<td>22</td>
</tr>
<tr>
<td>× 8</td>
<td>32</td>
</tr>
<tr>
<td>Totals</td>
<td>54</td>
</tr>
</tbody>
</table>

* Factor by which the MIC of trimethoprim was reduced in the presence of sulphamethoxazole.

There is some disagreement on the value of antibacterial treatment in Sonne dysentery, but if an antibacterial agent did shorten the period of excretion of the organism this might inhibit the spread of the disease. The results of in-vitro tests suggest that the combination of these drugs might be useful in the treatment of dysentery caused by sensitive strains of *Sh. sonnei*; not only does there appear to be potentiation between them, but it has been suggested that the use of the combination may postpone the emergence of organisms resistant to either component (Darrell, Garrod and Waterworth, 1968). The results cast some doubt on the possible value of including sulphonamide with trimethoprim in the treatment of infections with sulphonamide-resistant strains of *Sh. sonnei*. Bushby (1969) says that results of some preliminary tests suggest that sulphonamide does potentiate the bactericidal activity of trimethoprim against sulphonamide-resistant strains of *Escherichia coli* and that it also delays the emergence of trimethoprim resistance in these strains. He concludes that trimethoprim should seldom if ever be given without sulphonamide. A clinical trial would be necessary to establish the importance of these findings in the treatment of bacillary dysentery.

It is comforting to have another antibacterial agent to which all known strains of *Sh. sonnei* are sensitive when several strains—18 per cent. in this survey—were resistant to at least four of the commonly used agents; the only antibiotics to which we have never found strains of *Sh. sonnei* to be resistant are those of the polymyxin group. It is noteworthy
that 92 per cent. of the strains were resistant to ampicillin, despite the fact that ampicillin resistance does not usually appear to be transferable in Sh. sonnei (Scrimgeour).

SUMMARY

The minimum inhibitory concentrations of trimethoprim and sulphamethoxazole were determined for 209 strains of Shigella sonnei isolated mainly in the Greater London area. All strains were sensitive to trimethoprim at a concentration of 0.32 μg per ml, but 74 per cent. of strains were resistant to sulphamethoxazole at a concentration of 100 μg per ml.

Potentiation of trimethoprim by sulphamethoxazole was demonstrated in the case of sulphonamide-sensitive strains but there was little or no potentiation in the case of sulphonamide-resistant strains.

The possible value of the combination of trimethoprim and sulphamethoxazole in the treatment of Sonne dysentery is discussed briefly.

We thank Dr Joan R. Davies and Mr W. N. Farrant for providing many of the strains of Sh. sonnei and for performing the colicine typing.

REFERENCES

WATERWORTH, PAMELA M.
TOMLINSON, A. J. H.

A RAPID AND SIMPLE METHOD FOR THE LABORATORY DIAGNOSIS OF TRICHOPHYTON VERRUCOSUM

MARY P. ENGLISH

Pathology Laboratory, General Hospital, Guinea St, Bristol

Plates LV and LVI

The method of treatment of ringworm depends more on the site and type of the lesion than on the species of fungus causing the infection. For this reason the results of direct microscopy of a dermatological specimen in KOH, which can be given within half an hour, are all the clinician needs in order to decide whether or not to prescribe antifungal treatment. However, a fungus is sometimes overlooked on direct microscopy and is discovered only when cultures are made. Also, it is only by culturing and identifying the fungus that its probable source can be determined and steps be taken to prevent spread of the disease. Finally, in prognosis a knowledge of the identity of the fungus is essential. For these reasons, the rapid reporting of cultural results in dermatological mycology is very desirable.

Most laboratories use agar slopes, either in screw-capped bottles or, preferably, in plugged test-tubes, for the isolation of dermatophytes. In this laboratory petri dishes have always been used and have been found to have several advantages over slopes. Their use greatly

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