Resistance to trimethoprim in 1978-79 compared with 1973-75

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SUMMARY The incidence of resistance to trimethoprim among urinary isolates between October 1978 and November 1979 was 11.5%, more than double the figure found between April 1973 and October 1975. Of the resistant strains, 60% had a minimum inhibitory concentration in excess of 1 mg/ml. *Escherichia coli* and *Proteus mirabilis* showed the greatest increase in resistance since the previous study. Rather wide fluctuations occurred in the incidence of resistance for various species when the figures were analysed over two-month periods, hence studies of short duration must be interpreted with caution. There are not yet enough data from this or other studies for the cause of the increased incidence of resistance to trimethoprim to be determined.

During an 18-month period (April 1973 to October 1975) we surveyed the resistance to trimethoprim in Gram-negative bacteria isolated from infected urines at the Royal Free Hospital, London.¹ The incidence of resistance in hospital isolates was 3.2%.

In October 1978 we started another similar survey. It had originally been our intention to analyse the results after 18 months, in line with our previous study. However, two factors persuaded us to terminate the investigation in November 1979, that is, after 14 months. The factors were, firstly, the introduction for clinical use late in 1979 of trimethoprim alone and, secondly, the recent papers on the incidence of trimethoprim resistance from Finland² and Nottingham.³ We report our findings for 1978-79 and compare them with those we made in 1973-75.

Material and methods

All sensitivity plates from bacterial strains isolated in significant numbers from urines received for culture at this hospital were examined. Strains showing a reduced zone around the trimethoprim disc were then retested with an inoculum of 10⁴ colony-forming units per plate on Iso-sensitest agar (Oxoid) against discs of 1.25 µg and 2.5 µg trimethoprim. The minimum inhibitory concentration (MIC) of trimethoprim was then determined for strains appearing to be of diminished sensitivity.

Resistance was defined as an MIC of trimetho-

prim ≥ 4 µg/ml for *Proteus* spp. and ≥ 2 µg/ml for other bacterial species. These figures are based on data obtained previously,⁴ in which a statistical analysis was carried out on many fully sensitive strains in order to determine the upper limit of MIC for such populations.

Strains that were 'highly resistant' (MIC of trimethoprim > 1000 µg/ml) were tested for the ability to transfer their resistance to *Escherichia coli* K12. Biotyping of *E. coli* strains was carried out as described by Crichton and Old,⁵ the following characteristics being investigated: motility, ability to grow on minimal salts agar, decarboxylation of ornithine, and production of acid from rhamnose, raffinose, sorbose, and dulcitol. *Klebsiella* spp. were biotyped in terms of the following properties: decarboxylation of lysine, utilisation of citrate, production of arginine dihydrolase, urease, indole, gas from glucose, Voges-Proskauer reaction, and fermentation of sorbitol, sucrose, and arabinose.

Identification of all organisms causing urinary infections over the 14-month period was made in the routine laboratory with the API 20E and other appropriate systems.

Results

We investigated acquired resistance among the species listed in Table 1; these made up more than 80% of the strains isolated during the 14-month period.

Pseudomonads (401) and yeasts (205), being intrinsically resistant, were omitted from our study.

Table 1 Resistance to trimethoprim in bacteria isolated from urines between October 1978 and November 1979

Bacterial species	No. isolated	No. resistant	No. with MIC > 1 mg/ml	No. with transferable resistance
<i>Escherichia coli</i>	3641	259 (7.1%)	163	40
<i>Staphylococcus</i> spp.	522	137 (26.2%)	28	—
<i>Proteus mirabilis</i>	518	82 (15.8%)*	48	1
<i>Klebsiella</i> spp.	488	119 (24.4%)	59	24
<i>Micrococcus</i> spp.	167	3 (1.8%)	0	—
<i>Enterobacter</i> spp.	79	11 (13.9%)	10	5
<i>Citrobacter</i> spp.	47	15 (31.9%)	11	2

*For *Proteus* spp. the MIC was taken as ≥ 4 $\mu\text{g/ml}$ (see text).

Streptococci (641) were not studied because they had not been included in our previous survey and thus there was no baseline.

Of the 5462 strains in Table 1, 11.5% were resistant to trimethoprim. The highest incidence was among staphylococci, *Citrobacter* spp., and *Klebsiella* spp. The figure for *E. coli* strains was lower, being 7.1%, and that for micrococci was very low (1.8%).

As can be seen from Table 2, there were fluctuations in the incidence of resistance to trimethoprim during the study. The most remarkable variation was seen with staphylococci and *Proteus mirabilis*, which both doubled their incidence of resistance between December 1978/January 1979 and April/May 1979 but subsequently returned to their former values.

Most (60%) of the Gram-negative bacilli with acquired resistance were 'highly resistant' (Table 1). We succeeded in transferring resistance from almost half the resistant *Enterobacter* spp. and from a significant proportion of the *E. coli*, *Klebsiella*, and *Citrobacter* strains. On the other hand, transfer was observed from only one of the 48 resistant *Pr. mirabilis*. Overall, transfer of resistance was achieved from 24.7% of the highly resistant strains and from 14.8% of all resistant strains.

Twenty different patterns of transferable resistance were observed, of which the most common were: trimethoprim only (23), trimethoprim and streptomycin (12), and trimethoprim, ampicillin, chloramphenicol, and tetracycline (9).

We were able to subdivide 34 of the R⁺ *E. coli* strains into 12 biotypes, and 22 of the R⁺ *Klebsiella* (which comprised 18 *Klebsiella pneumoniae*, 3 *K. ozaenae*, and 1 *K. oxytoca*) into six biotypes.

Discussion

There has been a change for the worse in the situation since our last survey¹ of resistance to trimethoprim: then we found the overall frequency among *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Pr. mirabilis* to be 4.3%. Only 10% of those resistant strains were highly resistant. The present study shows that the incidence among these genera is now 10%, and 60% of these are now highly resistant. Resistance to trimethoprim mediated by R-factors is always associated with high-level resistance.⁶ Among the individual species, the figures for *Klebsiella* and *Enterobacter* spp. have remained virtually identical (contrary to our earlier prediction⁷), while for *Pr. mirabilis* and *E. coli* there have been increases of 7.5- and 5-fold, respectively. Trimethoprim alone became available only at the very end of our present survey period, and so our two sets of figures represent the situation while trimethoprim was available only in combination with a sulphonamide (usually sulphamethoxazole). Thus a definite increase in resistance has occurred since April 1975.

Our findings show that the incidence of resistance to trimethoprim at the Royal Free Hospital is lower for all species (except staphylococci) than that observed in Turku,² where trimethoprim alone has been widely used for several years. Unfortunately, however, these workers do not have a baseline with which to compare their present findings. For this reason, it is not possible, as the Turku authors point out, to say whether or not the greater resistance found by them is due to trimethoprim being used alone. Further, in an earlier study by Toivanen,⁸

Table 2 Variation in incidence of resistance to trimethoprim in various species, analysed at two-monthly intervals

Bacterial species	% resistant strains in two-month period						
	Oct/Nov 1978	Dec/Jan 1978/79	Feb/Mar 1979	Apr/May 1979	Jun/Jul 1979	Aug/Sep 1979	Oct/Nov 1979
<i>Escherichia coli</i>	8	5.5	7	9	7.5	11	11
<i>Staphylococcus</i> spp.	41	21	26	52	22.5	29	27.5
<i>Proteus mirabilis</i>	22	12.5	14.5	25.5	14.5	24	23
<i>Klebsiella</i> spp.	35.5	30	29	26	24	23	23.5

also from Turku, which immediately preceded his 1980 report,² 50% of the patients were catheterised, 73% were bed-ridden, and their average age was 73 years. If the same population was sampled for the later report² the high incidence of trimethoprim resistance (39.8%) is not surprising, as selection pressure is always heavy, and cross-infection common, under these conditions.

The large number of biotypes (18 from 56 strains) we found among the R⁺ *E. coli* and *Klebsiella* spp. strains argues strongly against cross-infection. There remains the possibility that one R-factor has spread throughout the hospital into many different hosts, but against this is the fact that we observed 20 different patterns of transferable resistance.

It is also worth stressing that even our increased figures are not greater than in previous reports from this country, for example, 8-10.8% at the Whittington Hospital⁹ in 1975 and 1977, with a 10-13% incidence of R-factor mediated resistance, and are much lower than in several reports from the continent of Europe, for example, 24-28% in Zurich,¹⁰ 17% in Paris,¹¹ and 20.3% in Pavia.¹²

In a separate communication¹³ we report on differences in trimethoprim resistance between hospital isolates and bacteria isolated from patients at home; in agreement with the Turku study,² we found the incidence of resistance to trimethoprim in *klebsiella* to be the same for inpatients and outpatients.

Recently, an interesting paper has appeared from workers at Nottingham.³ A point they stress, and with which we concur, is that the incidence of high-level, non-transferable resistance has increased recently. On the other hand, we find an increase also in all levels of resistance, which they do not. There are certain important differences between the studies, however. Firstly, the Nottingham workers examined all consecutive isolates from their hospital, while we confined ourselves to those from urinary infections; secondly, they took an arbitrary breakpoint of 8 µg/ml to indicate resistance.

The question of variation in breakpoint highlights the need not only for standardisation in this field (recently published breakpoints range from 8 to 1 µg/ml^{1-3,14}) but also for a more rational disc strength for use when testing urine pathogens against trimethoprim. The current practice of using a disc of 1.25 µg is clearly unsuitable in view of the known urine concentrations of trimethoprim during therapy, which are at least 50 µg/ml.^{15,16} We suggest that 10 µg discs be used in a trial to correlate laboratory results with clinical response.

Our results indicate the importance of continuing surveys for an adequate length of time, such as a

minimum of six months. Shorter periods may give a highly misleading sample. Thus, this must cast doubt on several studies: for example, Huovinen and Toivanen² and Amyes *et al.*⁹ carried out studies for three months or less. If an R-factor appeared, spread, and disappeared¹⁷ in a short period, and the sample happened to be taken at the peak, the findings would lead to incorrect conclusions.

As we have pointed out elsewhere,¹⁸ there is a surprising lack of satisfactory information on the occurrence of resistance to trimethoprim in different places. A widely used approach to sensitivity patterns is the compilation of 'league tables'^{19,20} showing sensitivities of consecutive isolates from a laboratory to several antibiotics. Although this information is extremely useful for certain purposes, it can be misleading when long-term trends in trimethoprim resistance are considered. Problems arise when outbreaks of infection due to naturally resistant bacteria or cross-infection involving trimethoprim-resistant organisms¹⁷ occur. In such cases, 'league tables' may give false information to those who prescribe antimicrobial agents because they give an overestimate of the incidence of resistance.

Now that trimethoprim alone has been marketed in the United Kingdom a turning point has been reached in the continuing debate concerning the merits of using trimethoprim alone or only in combination with a sulphonamide. Clinical trials in urinary infections have so far failed to show any advantage of the combination over the monosubstance.²¹ Thus, one of the original justifications for the use of the combination (therapeutic synergy) has been proved groundless, at least for urinary infections. The way now seems open for trials in other indications, such as chest infections.¹⁴ The second justification for the use of the combination was that this would prevent, or at least retard, the emergence of resistance. The experience in Finland is not decisive—in Turku resistance is relatively common² (although as there is no baseline it cannot be said for certain that it has increased) while in Helsinki¹⁵ it is rare despite trimethoprim alone having been used throughout the country for some time. Our own findings¹ (and present communication) indicate that resistance to trimethoprim has increased despite the use of the combination only. Because both the combination and the monosubstance will now be in use at the same time, this will make the solution to this problem even more difficult. However, regular, detailed surveys to determine resistance to both the monosubstance and combination (trimethoprim and sulphamethoxazole) will be an essential part of the process of gathering evidence.

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