THE SUSCEPTIBILITY OF 168 STRAINS OF SALMONELLAE TO ELEVEN ANTI-MICROBIAL AGENTS

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Salmonellae are generally sensitive to a number of anti-microbial agents in vitrio. Yet only a few drugs have been found to give satisfactory results clinically. Both chloramphenicol and ampicillin have been in use for several years against S.typhi, but the problem of typhoid carriers is still a serious one. The search for new alternative drugs have not, on the whole, been fruitful. The appearance of the new combination, consisting of trimethoprim and sulphamethoxazole, has led us to compare its effectiveness to ten other drugs available.

MATERIALS AND METHODS

Strains: 168 strains were isolated by the bacteriology laboratory from July 1968 to March 1969. These were primary isolates from routine specimens, all of which came from different patients.

Identification: Serotypes were identified using antisera (Burroughs Wellcome) and the Kauffman-White Schema.

Media: The following antibiotics were incorporated into aliquots of DST (Oxoid) agar, which have been melted and cooled, to give concentrations of 25, 12·5, 6·2, 3·2, 1·6 and 0·8 µg./ml.: ampicillin, cephaloridine, tetracycline, chloramphenicol, streptomycin, kanamycin, neomycin, gentamicin and polymyxin. Sulphadiazine concentrations were 100, 50, 25 and 12·5 µg./ml. Trimethoprim (lactate form supplied by Burroughs-Wellcome) was combined with sulphadiazine in the ratio of 1:20 to give concentrations from (0·8+16) to (0·0008+0·016) µg./ml. in doubling dilutions. The batches of media were then poured on to petri dishes, and allowed to set.

Assay: 0·01 ml. of 10^{-2} dilution in sterile normal saline of an overnight broth culture of each strain was dropped on to each plate from a 100 dropper. By means of a template, it was possible to test 36 to 40 strains on each plate. The plates were incubated at 37°C overnight. The highest dilution of drug at which no growth was visible was taken as the minimal inhibitory concentration (MIC). Tests involving sulphadiazine and trimethoprim had inocula taken from 10^{-4} dilutions of overnight cultures, and the highest dilution that brought about approximately 90% growth inhibition was taken as the MIC.

RESULTS

There were 104 strains of S. typhi and 64 other salmonellae (Table I). Of the latter, most of them belonged to Group B.S. derby, S. typhimurium and S. paratyphi B. accounted for 39 of the 64 strains.

TABLE I

SEROTYPES OF SALMONELLAE STUDIED

Group	Serotype	Number
D	S. typhi	104
A	S. paratyphi A	2
B	S. paratyphi B	9
	S. abony	1
	S. bredeney	1
	S. derby	18
	S. typhimurium	12
	S. chester	1
C	S. choleraesuis (var. kunzendorf)	1
	S. hindmarsh	1
	S. newport	2
	S. bovismorbificans	2
	S. tennessee	2
	S. oranienburg	1
D	S. enteritidis	1
E	S. anatum	2
	S. nchanga	2
	S. weltevreden	6
	TOTAL	168

Fig. 1 shows that at a concentration of 6·2 µg./ml. most strains of S. typhi could be inhibited by ampicillin, cephaloridine, chloramphenicol and tetracycline. At this concentration chloramphenicol was able to inhibit all strains. Against most strains the drug was approx-

imately twice as effective as tetracycline, which inhibited 95·3% at 6·2 μg./ml. One strain was resistant to 25 μg./ml. tetracycline. Ampicillin and cephaloridine were approximately equally effective. Two strains had MICs of 12·5 μg./ml. against ampicillin, whilst one had a MIC of 12·5 μg./ml. and another 25 μg./ml. against cephaloridine. Against over 90% of the strains, both drugs were slightly more effective than chloramphenicol.

The non-S. typhi strains were generally less sensitive than the S. typhi strains against the four drugs (Fig. 2). This was particularly obvious at low concentrations of the drugs. Chloramphenicol was again more effective than tetracycline, and was able to inhibit all strains at 12·5 μg./ml. One S. derby strain was resistant to 25 μg./ml. tetracycline. Cephaloridine was slightly more effective than ampicillin. One S. choleraesuis strain had a MIC of 25 μg./ml. for cephaloridine, whilst one S. paratyphi B was resistant to 25 μg./ml. ampicillin. Except for these resistant ones, most strains were just as sensitive to cephaloridine and ampicillin as they were to chloramphenicol.

Amongst the aminoglycosides (Figs. 3 and 4), gentamicin was the most effective, whilst streptomycin the least active. All were susceptible to 3·2 μg./ml. gentamicin, whilst one S. typhi, one S. typhimurium, two S. weltevreden and five S. derby were resistant to 25 μg./ml. streptomycin. The same S. typhi strain was also resistant to 25 μg./ml. kanamycin and neomycin. Although neomycin was slightly more active than kanamycin, the difference was minimal. Only six of the 168 strains had a difference of two doubling dilutions in the MICs for the two drugs. The rest had not more than one doubling dilution difference. One S. abony strain was resistant to 25 μg./ml. kanamycin and neomycin, but sensitive to 6·2 μg./ml. streptomycin.

Polymyxin (Fig. 5) was highly effective, and all strains were sensitive to 3·2 μg./ml. Here again, S. typhi proved to be more sensitive than the other salmonellae.

Resistance to sulphadiazine was found in a number of strains (Fig. 6). Two S. typhi and fourteen of the other salmonellae were resistant to 100 μg./ml. Seven of the eighteen S. derby were insensitive. On the whole, S. typhi strains were two to four times more sensitive than the other salmonellae.

The trimethoprim/sulphadiazine combination (Fig. 6) in the ratio of 1:20 was very effective against S. typhi. All the strains were

TABLE II

THE SENSITIVITY OF SALMONELLAE TO VARIOUS ANTIMICROBIAL AGENTS

Drugs	S. typhi	non S. typhi salmonellae
Ampicillin (12·5 μg./ml.)	100%	98·4%
Cephaloridine (12·5 μg./ml.)	99%	98·4%
Chloramphenicol (12.5 μg./ml.)	100%	100%
Tetracycline (6·2 μg./ml.)	95·3%	90·6%
Gentamicin (3·2 μg./ml.)	100%	100%
Neomycin (12·5 μg./ml.)	99%	98·4%
Kanamycin (12·5 μg./ml.)	99%	98·4%
Streptomycin (12·5 μg./ml.)	97·1%	85·9%
Polymyxin (3·2 μg./ml.)	100%	100%
Sulphadiazine (100 μg./ml.)	98·1%	78·1%
Trimethoprim/ sulphadiazine	100% (1·05μg./ml.)	100% (16·8μg./ml.)

TABLE III

RESISTANCE OF SEROTYPES TO ANTIMICROBIAL AGENTS

Serotypes	Ampicillin (12·5 ug./ml.)	Cephaloridine (12·5 ug./ml.)	Tetracycline (6·2 ug./ml.)	Streptomycin (12·5 ug./ml.)	Neomycin (12·5 ug./ml.)	Kanamycin (12·5 ug./ml.)	Sulphadiazine (100 ug./ml.)
S. typhi		1	5	3	1	1	2
S. paratyphi B	1			1			3
S. abony					1	1	
S. derby			1	5			7
S. typhimurium			4	1			1
S. hindmarsh							1
S. bovismorbificans							1
S. choleraesuis		1					
S. tennessee			1				
S. weltevreden				2			1

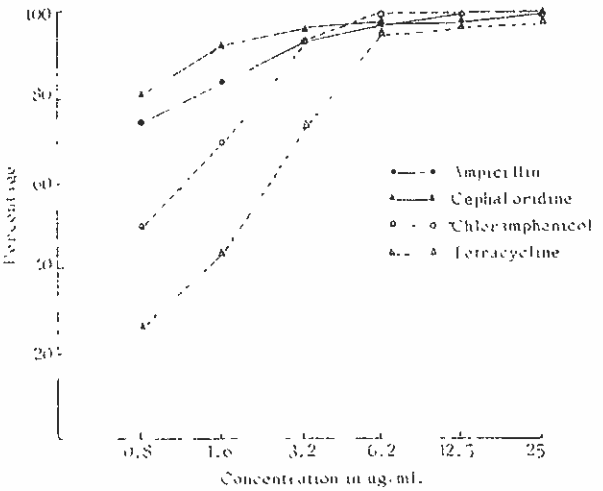

Fig. 1. Sensitivity of S. typhi.

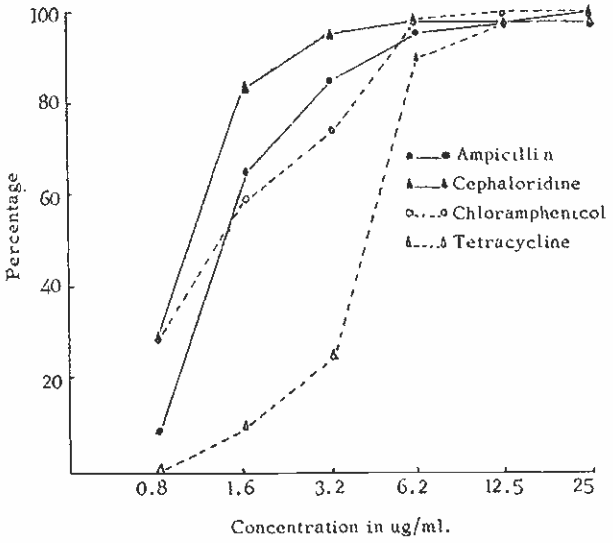

Fig. 2. Sensitivity of non-S. typhi Salmonellae.

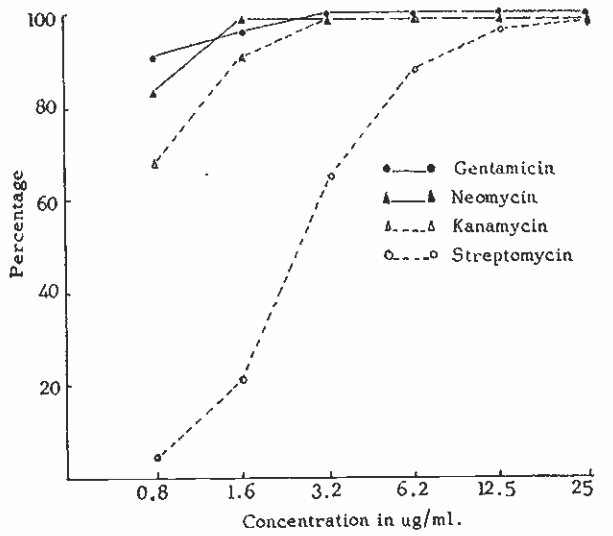

Fig. 3. Sensitivity of S. typhi.

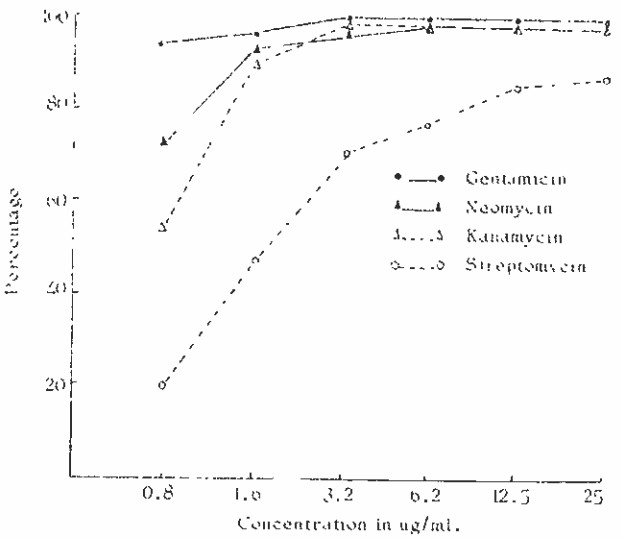

Fig. 4. Sensitivity of non-S. typhi Salmonellae.

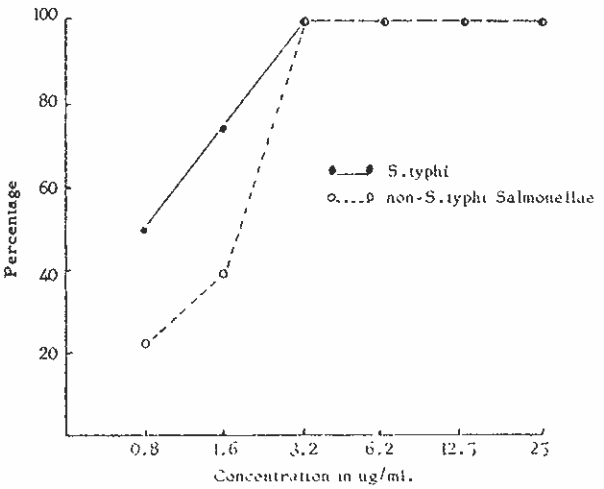

Fig. 5. Sensitivity of Salmonellae to Polymyxin.

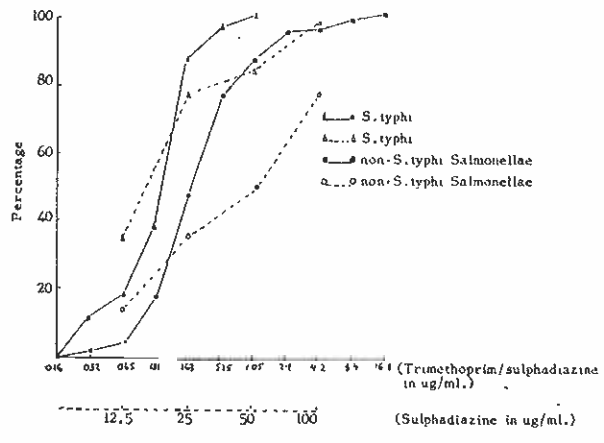

Fig. 6. Sensitivity of Salmonellae to Trimethoprim and Sulphadiazine.

susceptible to 1·05 µg./ml. At this concentration, 82·2% of the non-S. typhi strains were sensitive. The least sensitive was one S. cholerasuis which had a MIC of 16·8 µg./ml.

Table II gives the percentages of strains sensitive to the various drugs at the concentrations shown. S. typhi strains were very susceptible to the drugs used. The most effective were trimethoprim/sulphadiazine, gentamicin, polymyxin, chloramphenicol and ampicillin. Against the non-S. typhi strains the most effective drugs were gentamicin, polymyxin, chloramphenicol and trimethoprim/sulphadiazine.

Table III shows the serotypes resistant to the various drugs. S. typhimurium accounted for most of the tetracycline-resistant strains, and S. derby were on the whole fairly resistant to streptomycin and sulphadiazine.

DISCUSSION

Despite the sensitivity of S. typhi to a wide variety of antibiotics, as shown in our study, only ampicillin and chloramphenicol have proved to be value clinically. This illustrates the difficulty in assuming clinical usefulness of antimicrobial drugs in the treatment of typhoid solely from laboratory tests.

Fortunately, strains resistant to the two drugs are infrequent. In our series all were sensitive to 12·5 µg./ml. ampicillin and 6·2 µg./ml. chloramphenicol.

Cephaloridine was as effective as ampicillin, though one strain had a MIC of 25 µg./ml. However, its use in the treatment of typhoid fever has not been encouraging (Hermans *et al*, 1966).

Gentamicin and polymyxin were effective against all strains at 3·2 µg./ml. In view of their toxicity, their clinical value is limited.

The new drug combination trimethoprim/sulphonamide seems to offer promise. 1·05 µg./ml. of the combination inhibited the growth of all S. typhi. This was approximately three times the effectiveness of gentamicin and polymyxin, which required 3·2 µg./ml. for similar effect, and six times that of chloramphenicol, which inhibited all strains at 6·2 µg./ml. In one clinical trial favourable results were reported (Akinkugbe *et al*, 1968). Larger series will need to be studied to assess its effect on the carrier rate.

Salmonella serotypes other than those causing enteric infections generally give rise to infections limited to the gut. The usefulness of antimicrobial agents in the treatment of uncomplicated salmonella enteritis has been questioned recently (Editorial, Brit. Med. J., 1969). There is evidence (Aserkoff and Bennett, 1969) that patients given antibiotics take longer to clear the organisms from their intestines; and, what is even more disturbing, in 97% of them the strains acquired antibiotic resistance, whereas none of those untreated excreted resistant strains.

Certain serotypes, however, are liable to cause systemic infections. One such strain is S. choleraesuis, which causes septicaemia in almost 50% of patients (Saphra and Winter, 1957). As the mortality is in the region of 20%, it is an organism to be taken seriously. Our single strain of S. choleraesuis was fairly resistant to cephaloridine (MIC 25 µg./ml.), and trimethoprim/sulphadiazine (MIC 16·8 µg./ml.). Fortunately, it was sensitive to ampicillin (MIC 1·6 µg./ml.) and chloramphenicol (MIC 6·2 µg./ml.).

The non-S. typhi strains were generally less sensitive than the S. typhi. This was especially apparent with sulphadiazine and trimethoprim/sulphadiazine.

There were some differences in the sensitivity patterns of the individual serotypes. Four (33·3%) of the S. typhimurium were resistant to 6·2 µg./ml. tetracycline, contrasting with the 9·4% resistance shown by all non-S. typhi strains. The percentages of S. derby resistant to 12·5 µg./ml. streptomycin (27·8%) and 100 µg./ml. sulphadiazine (38·9%) were also higher than what was found for the other strains, which was 14·1% and 21·9% respectively.

Despite the decreased sensitivity, there was no lack of effective antimicrobial agents against the salmonellae serotypes. All were sensitive to 6·2 µg./ml. chloramphenicol, and only one S. paratyphi B was resistant to 25 µg./ml. ampicillin. Cephaloridine was also effective, though one S. choleraesuis had a MIC of 25 µg./ml. Trimethoprim/sulphadiazine at 16·8 µg./ml. inhibited all strains. This level is attainable in the serum after normal dosage (Bushby and Hitchings, 1968), and the drug may have a place in the treatment of systemic salmonellosis.

Kanamycin and neomycin were effective against 63 of the 64 strains. Though toxic, they are almost wholly non-absorbed given orally, and their usefulness would be limited to the gut.

Tetracycline, streptomycin and sulphadiazine were not as effective, and their use should best

be withheld until sensitivity tests show strains to be susceptible.

SUMMARY

168 strains of salmonellae were tested for sensitivity to eleven antimicrobial agents. The drugs most effective against S. typhi were trimethoprim/sulphadiazine, gentamicin, polymyxin and chloramphenicol. Against most strains ampicillin and cephaloridine were also active. Trimethoprim/sulphadiazine was approximately three times as active as gentamicin and polymyxin, and six times that of chloramphenicol.

The non-S. typhi strains were comparatively less sensitive, especially against sulphadiazine and trimethoprim/sulphadiazine. Nonetheless, the latter at a concentration of 16·8 µg./ml. was effective against all strains. Gentamicin and polymyxin at 3·2 µg./ml. and chloramphenicol at 6·2 µg./ml. inhibited all strains. Ampicillin and cephaloridine compared favourably with chloramphenicol, except for a few strains.

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