LABORATORY EVALUATION OF ERYTHROMYCIN IN A GENERAL HOSPITAL*

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The present therapeutic use of erythromycin has tended to be limited, mainly because of historical accident. First introduced when penicillin-resistant staphylococcal infections were becoming a problem, its use was restricted in many areas to the control of such infections and discouraged in treatment of other infections, because of possible danger of emerging bacterial resistance. Adequate control of staphylococci resistant to benzylpenicillin is now available with other antibiotics, but in spite of this the therapeutic value of erythromycin in other disease large general hospital.

The present study was undertaken to determine current *in vitro* sensitivity patterns to erythromycin of organisms isolated from material submitted from the wards of a large general hospital.

METHODS

Disc Sensitivity Tests

Tests were carried out with standard 15-μg. dried discs. In general the methods of Petersdorf and Sherris[1] were adopted, with certain minor modifications.

Inocula were prepared from 6-hour fluid cultures suitably diluted. The dilution chosen was that which resulted in almost confluent growth on agar plates after 16 hours' incubation. With practice good reproducibility of result can be obtained, but is easier to achieve with some organisms, e.g. staphylococci, than with others. Zone diameters were read after 16 hours' incubation at 37°C and were measured to include the 6-mm. diameter of the disc. Thus, a completely resistant organism is expressed as having a 6-mm. zone, i.e. that of the disc. Generally the edge of the inhibition zone was clearly defined, but, where necessary, several readings were made and the mean diameter was calculated.

Minimum inhibitory concentrations were estimated in suitable fluid culture media by standard methods.[2] Again results were read after 16 hours' incubation at 37°C by visual inspection of the tubes.

RESULTS

Zone sizes are detailed in Table I. Organisms with zones of 13 mm. or less were considered as resistant, 14 - 17 mm. as intermediate and 18 mm. or greater as sensitive. For the enterobacteriaceae group, zone sizes of 10 - 13 mm. are also given in Table II for reasons mentioned below. Zones greater than 25 mm. are shown in Table I simply to demonstrate the high sensitivity of these organisms.

The above standards have been calculated from a correlation of MIC levels and zone sizes, and are similar to those of Petersdorf and Sherris.[1] Their standards apply essentially to the use of Mueller-Hinton medium. Not

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all our tests were done with this medium, but the variations introduced by the use of other media were not sufficient to influence interpretation of results significantly. For this reason the above standards have been applied to all disc tests.

TABLE I. ZONE SIZES WITH 15-μG ERYTHROMYCIN DISCS

Organism	*Zone sizes (mm.)*				
	0–13	*14–17*	*18–24*	*25+*	*Total*
Staph. pyogenes	115	15	187	297	614
Staph. saprophyticus	68	7	198	559	832
Strep. viridans	0	0	18	32	50
β-haemolytic streptococcus	0	3	35	20	58
Non-haemolytic streptococcus	1	2	12	19	34
Strep. faecalis	2	6	11	0	19
Anaerobic streptococcus	0	0	12	30	42
Pneumococci	0	1	13	17	31
Neisseriae	1	5	28	25	59
Corynebacteria	0	3	37	109	149
Esch. coli	1,324	16	4	0	1,344
'Paracolon'	47	3	0	0	50
Salmonella	37	3	0	0	40
Klebsiella	66	1	0	0	67
B. faecalis alkaligenes	35	8	0	0	43
Proteus	69	0	0	0	69
Pseudomonas	98	0	0	0	98
Mimea	8	8	31	10	57
Haem. influenzae	0	8	2	25	35

TABLE II. ZONE SIZES FOR ENTEROBACTERIACEAE AND PSEUDOMONAS STRAINS

Organism	*Zone sizes (mm.)*					
	0–9	*10–13*	*14–17*	*18–24*	*25+*	*Total*
Esch. coli	1,150	174	16	4	0	1,344
'Paracolon'	28	19	3	0	0	50
Salmonella	31	6	3	0	0	40
Klebsiella	53	13	1	0	0	67
B. faecalis alkaligenes	26	9	8	0	0	43
Proteus	69	0	0	0	0	69
Pseudomonas	98	0	0	0	0	98

Comparison of zone sizes with MIC is necessary if the former are to be interpreted intelligently in terms of probable clinical efficacy. For this reason it is often impossible to assess results where organisms are simply reported as 'sensitive', since the zone sizes are not stated and the concentrations of antibiotics at the boundaries of inhibition zones are unknown. Regression curves prepared from this type of data are suitable for use with erythromycin since they have neither the steep slopes given by polypeptide antibiotics nor the flat, almost horizontal curve given by cycloserine.

Enterobacteriaceae Group

Erythromycin has generally been considered as being of no value against Gram-negative intestinal organisms. Reports to the contrary include that of Williamson and Zinnemann[3] and a recent study from a South of England teaching hospital.[4] The latter report indicated that 4 of 10 (40%) 'coliforms' were sensitive to erythromycin, as well as 63 of 159 (39·6%) 'atypical' coli strains and 111 of 894 (12·4%) *Esch. coli* strains. In neither report is sensitivity defined and in the latter the discs used contained 50 μg. of erythromycin. For these reasons it is of interest to compare Tables III and IV. Table III shows percentage sensitivity for this group by the standards defined above, and Table IV shows the results obtained if any zone of inhibition is considered as indicating sensitivity. The results in Table IV are, of course, quite misleading in terms of

TABLE III. PERCENTAGE SENSITIVITY OF ENTEROBACTERIACEAE AND PSEUDOMONAS STRAINS *

Organism	*No. sensitive/ No. tested*	*% sensitive*
Esch. coli	20/1,344	1·4
'Paracolon'	3/50	6·0
Salmonella	3/40	7·5
Klebsiella	1/67	1·4
B. faecalis alkaligenes	8/43	18·6
Proteus	0/69	0·0
Pseudomonas	0/98	0·0

* Greater than 13 mm. diameter.

TABLE IV. PERCENTAGE SENSITIVITY OF ENTEROBACTERIACEAE AND PSEUDOMONAS STRAINS *

Organism	*No. sensitive/ No. tested*	*% sensitive*
Esch. coli	194/1,344	14·4
'Paracolon'	22/50	44·0
Salmonella	9/40	22·5
Klebsiella	14/67	20·8
B. faecalis alkaligenes	8/43	18·6
Proteus	0/69	0·0
Pseudomonas	0/98	0·0

* Any zone of inhibition.

clinical usefulness. Tube dilution methods showed that the MIC for the 7 - 13-mm. zone diameter organisms lay between 12·5 and 100 μg./ml., and occasionally much higher. Since these levels are not attained on standard dosage regimens (except in urine), such organisms should be reported as resistant. This is possibly not done since some technologists appear to believe that any zone of inhibition indicates 'sensitivity'. In fact this only applies to antibiotics with poor diffusibility such as colistin, kanamycin, polymyxin, bacitracin and ristocetin.

The selection of 13 mm. is, of course, arbitrary and interpretation of results in the intermediate zone of 14 - 17 mm. can present difficulties. Ideally, MIC levels should be established, but this creates problems in a busy laboratory. A comparison of the results in Tables III and IV illustrates clearly the need to correlate these parameters if laboratory findings are to be reliable indicators of potential therapeutic value.

Staphylococci

Of 614 coagulase-positive strains of *Staph. pyogenes*, 499 (81·2%) were sensitive. These findings are similar to those of other workers. Harris,[4] in the report referred to above, quotes a figure of 95·2% sensitivity of 1,112 strains. In the present series some 90% of the strains came from patients with hospital-acquired infections. A comparative study of 832 non-pathogenic strains of *Staph. saprophyticus* showed an over-all sensitivity of 91·8%.

It may be noted that of the 614 strains of *Staph. pyogenes*, only 15 fell into the intermediate range with zones between 14 and 17 mm. For this reason the interpretation of 'sensitivity' in this group was clear-cut.

Mimea Group

An interesting observation was made early in the investigation that a number of strains initially thought to

be *B. faecalis alkaligenes* on the basis of biochemical reactions, were very sensitive to erythromycin. Further investigation revealed that they were in fact mimea strains. The majority were isolated from urinary tract infections. Their sensitivity to erythromycin has become a useful routine method for the preliminary identification of such strains.

Corynebacteria

Of the 149 strains in this group, 25 were toxigenic strains of *Corynebacterium diphtheriae*. Strains were uniformly sensitive to erythromycin.

Salmonella

In addition to the strains shown in Table I, 50 stock culture strains of *S. typhi* were tested. In each case zone sizes were in the 7 - 13-mm. range and the MIC was correspondingly high, i.e. greater than 25 μg./ml.

TABLE V. PERCENTAGE SENSITIVITY OF OTHER ORGANISMS

Organism	*No. sensitive/ No. tested*	*% sensitive*
Staph. pyogenes	499/614	81·2
Staph. saprophyticus	764/832	91·8
Strep. viridans	50/50	100
β-haemolytic streptococcus	58/58	100
Non-haemolytic streptococcus	33/34	97·7
Strep. faecalis	17/19	89·4
Anaerobic streptococcus	42/42	100
Pneumococci	31/31	100
Neisseriae	58/59	98·3
Corynebacteria	149/149	100
Mimea	49/57	85·9
Haem. influenzae	35/35	100

Other groups showed the patterns expected (Table V). Of the neisseriae group, one strain of *N. meningitidis* was resistant. In the case of *Haem. influenzae* we had anticipated that zone sizes would be small since MIC levels for this organism are usually about 2·5 μg./ml. However, this was not so, 23 of the 25 strains having zone sizes of 25 mm. or greater.

When zone sizes were correlated with the percentage of strains observed for each zone, different types of curves could be plotted. Both *Staph. pyogenes* and *Staph. saprophyticus* showed a bimodal curve, as did *Haem. influenzae*. The remaining groups showed unimodal distribution with varying degrees of spread of the curve. Thus, klebsiella species show a sharp curve of resistance, whereas corynebacteria, haemolytic streptococci, non-haemolytic streptococci, *Strep. faecalis*, neisseriae and mimea strains show widespread unimodal curves. On the other hand, *Strep. viridans* strains show a much more restricted unimodal curve.

The correlation between zone size and MIC was good and only occasional exceptions were noted. The reasons for this are not clear but exceptions to both types were found, i.e. (*a*) a small zone size with a low MIC, and (*b*) a large zone size with an unexpectedly high MIC. Such variations may be due to technical faults, but repeated investigations show that this is not always the reason and the observation requires further study.

DISCUSSION

The place of erythromycin in therapeutics is generally considered to be limited, partly because more effective bactericidal drugs are available to treat penicillin-resistant staphylococcal disease. However, in neonatal staphylococcal infection Forfar *et al.*[5] found that although erythromycin did not reduce the incidence of infection, it did, when used in minor staphylococcal infection, significantly reduce staphylococcal mortality and osteomyelitis rates. However, these workers also noted that in the presence of apparent erythromycin resistance there appeared to be no correlation between the results of sensitivity tests and clinical effectiveness of erythromycin.

Mouratoff *et al.*[6] noted a different type of lack of correlation when erythromycin was used to treat chronic urinary tract infections. Clinical improvement was observed in spite of a failure of bacteriological improvement, even though the infecting organisms appeared sensitive *in vitro*. Thus, of 10 such patients bacteriological control was established in only 3. However, in 14 patients where the infecting organisms were resistant to erythromycin *in vitro*, 12 showed clinical improvement without any bacteriological improvement.

It is usually considered that erythromycin is of no value in management of Gram-negative bacillary infections. However, Williamson and Zinnemann[3] found that 53% of 347 strains of coliform organisms were sensitive when tested with 10 μg. of erythromycin in punch-plates, whereas poor results were obtained with discs or with impregnated tablets. They concluded that a proportion of urinary tract infections may usefully be treated with erythromycin, especially where resistance to other antibiotics is a problem. Such use of erythromycin may be justified, since concentrations in excess of 1,000 μg./ml. can be obtained in urine with dosage regimens of 500 mg. every 6 hours. Additionally, alkalinization of the urine will increase erythromycin activity, and Zagar[7] has shown that MIC levels may be reduced 50 - 100-fold at pH 8·4 as compared with pH 6·4.

Erythromycin is an antibiotic which should be considered more often by clinicians than is currently the case. Its virtual lack of toxicity, apart from the danger of cholestatic hepatitis caused by the estolate derivative, and low rate of hypersensitivity induction make it a drug of considerable value in many common therapeutic situations.

SUMMARY

Current *in vitro* patterns of sensitivity to erythromycin were investigated for a number of common pathogenic organisms isolated from patients in a large general hospital.

The findings, particularly in the enterobacteriaceae group, again stress the need for correlation of sensitivity zones with tube MIC in interpretation of the former.

The status of erythromycin as a reserve drug is no longer justified and its more general use in place of other more toxic antibiotics deserves closer consideration by clinicians.

REFERENCES

1. Petersdorf, R. G. and Sherris, J. C. (1965): Amer. J. Med., **39**, 766.
2. Cruickshank, R. (1965): *Medical Microbiology*, 11th ed. Edinburgh: E. & S. Livingstone.
3. Williamson, G. M. and Zinnemann, K. (1961): Antibiot. and Chemother., **12**, 169.
4. Harris, E. L. (1968): Personal communication.
5. Forfar, J., Keay, A. J., Maccabe, A. F., Gould, J. C. and Bain, A. D. (1966): Lancet, **2**, 295.
6. Mouratoff, G., Bell, M. and Batterman, R. C. (1956-57): *Antibiotics Annual*, p. 159. New York: Medical Encyclopedia Inc.
7. Zagar, Z. (1963): Chemotherapia (Basel), **6**, 82.