

The *in vitro* drug resistance of *Salmonella dublin* isolated from cattle

BY M. HINTON* AND B. M. WILLIAMS†

Veterinary Investigation Centre, Carmarthen, S. Wales

(Received 24 February 1977)

SUMMARY

The resistance of *Salmonella dublin* to eight antibacterial drugs was assessed under diagnostic laboratory conditions. Two techniques were used consecutively and the differences in the results are described. Strains of *S. dublin* isolated from either calves, adult cattle or abortion material showed no differences in resistance for either of the techniques, though differences between the two techniques were found.

INTRODUCTION

As an adjunct to an investigation into bovine abortion associated with *Salmonella dublin* infection (Hinton, 1974) the drug resistance of strains of *S. dublin* isolated from calves, adult cattle and abortion material was assessed. Two techniques for sensitivity testing were used during the survey period and the differences in the results obtained are described in this paper.

MATERIALS AND METHODS

All the *S. dublin* strains were isolated during the course of routine diagnostic investigations and were derived from three main sources, namely calves (faecal swabs and viscera), adult faeces (swabs and faecal samples) and abortion material (fetal stomach, fetal membranes or vaginal mucous swabs). A total of 406 strains were examined using the disk diffusion method.

Diagnostic sensitivity test agar base (Oxoid Ltd) was used throughout this investigation. In the first part of the survey, between September 1969 and June 1971, organisms from a selected colony were taken on a loop and spread on the medium which was then overlaid by 'high' level Multodisk (Oxoid 1334E). In 1971 a standard testing procedure was introduced into the whole of the Veterinary Investigation Service. Organisms were taken as for the first method and then were emulsified in 3 ml peptone water. Three drops of the suspension, from a Pasteur pipette, were spread on the surface of the medium. This regularly produced a nearly confluent growth. The sensitivity of the test was also increased by using a 'low' level Multodisk (Oxoid 1332E) in which the quantity of the drug in seven of the

* Present address: Department of Veterinary Medicine, University of Bristol, Langford House, Langford, Nr. Bristol. BS18 7DU.

† Present address: M.A.F.F., State Veterinary Service, Hook Rise South, Tolworth, Surbiton KT6 7NF.

Table 1. *The antibiotic resistance of Salmonella dublin strains isolated from cattle using two different methods of testing*

Method	Source of strain	No. strains	HLD†	LLD‡	Antibiotic disk code (Oxoid)*													
					A	C	FR	N	P	PN	S	S3						
Old technique High level disk (Oxoid 1334E)	Calves	86			4	1	1	0	61	5	43	64						
	Adults	116			1	0	3	0	73	5	61	91						
	Abortions	52			0	0	0	0	29	2	29	50						
	Total	254			5	1	4	0	163	12	133	205						
New technique Low level disk (Oxoid 1332E)	Calves	33			0	0	0	0	31	1	31	21						
	Adults	53			0	0	0	0	44	3	42	21						
	Abortions	66			0	0	0	0	57	6	52	31						
	Total	152			0	0	0	0	132	10	125	73						
Comparison between the techniques																		
					χ^2	6.2				23	0.6	37	47					
					P	<0.02				<0.01	NS	<0.001	<0.001					

* A = chlortetracycline, C = chloramphenicol, FR = furazolidone, N = neomycin, P = penicillin, PN = ampicillin, S = streptomycin, S3 = sulphonomide compound.

† High level disk (μ g). ‡ Low level disk (μ g). § Units of penicillin.

Table 2. *The resistance of Salmonella dublin to spectinomycin*

Source of strain	25 μ g disk		50 μ g disk	
	No. examined	No. resistant	No. examined	No. resistant
Calves	15	3	7	0
Adults	30	0	12	0
Abortions	37	5	17	0
Total	82	8	36	0

eight disks was reduced. In addition to this a limited number of strains were also tested against spectinomycin using 25 and 50 μ g disks.

The plates were incubated at 37 °C in air and were examined at the end of the working day or if there was insufficient growth, the next morning. The results were scored as either sensitive or resistant with a zone of < 2 mm width being taken as resistant.

RESULTS

The main results are listed in Table 1. When the two methods used for the testing of drug resistance are considered separately, a comparison of the results for the strains of *S. dublin* isolated from either calves, faeces or abortions revealed only minor and statistically non-significant differences in the number of strains resistant to the individual drugs.

A comparison of the total results for each of the techniques indicated that the introduction of the second method led to a significant increase ($P < 0.001$) in the number of strains resistant to penicillin and streptomycin. The proportion rose from 64 to 87% and 52 to 87% respectively. The resistance to compound sulphonamides decreased significantly ($P < 0.001$) from 84.5 to 48%. There was also a reduction ($P < 0.02$) in the combined results from resistance to chlortetracycline, chloramphenicol and furazolidone.

The findings in the smaller trial involving spectinomycin are listed in Table 2. Resistant strains were only found when the 25 μ g disk was used, though this difference was not statistically significant. At 25 μ g spectinomycin appeared similar to, but not quite as effective as, 2 μ g ampicillin.

DISCUSSION

The results obtained using the disk diffusion technique are influenced by a number of factors including the composition of the medium, the quantity and diffusibility of the drug in the disk, the size of the inoculum and the time of incubation (Garrod & O'Grady, 1971).

The differences in the results obtained using the two techniques may reflect a change in the resistance pattern of the field strains of *S. dublin* though it is unlikely that such a dramatic change would have occurred in such a short time. This means that most of the differences observed were due to the change in technique. The reduction in resistance of *S. dublin* to chlortetracycline, chloramphenicol and furazolidone is difficult to explain but suggests that the size of the

inoculum, which was reduced, is of greater importance in determining resistance than the concentration of the drug in the disk which was also reduced from 50 to 10 μg . On the other hand the changes in resistance to penicillin, streptomycin and compound sulphanamide are more likely to reflect changes in the concentration of the drugs in the disk, though White & West (1974) indicate that a reduction in density of the growth will also be associated with a decrease in sulphonamide resistance.

This survey was carried out before trimethoprim/sulphonamide had become a well-established veterinary therapeutic agent, and so it was not included in the testing programme. Nevertheless Sojka, Hudson & Slavin (1974) and Sojka & Hudson (1976) report favourably on this drug combination in that only 6 (0.0013%) of 4368 salmonella strains tested were resistant.

The conclusions that can be drawn from this survey are first that there were no significant differences in the resistance of *S. dublin* strains isolated from either calves, adult faeces or abortions, and secondly the degree of resistance to the important therapeutic drugs, namely chlortetracycline, chloramphenicol, furazolidone and neomycin is of a very low order and the demonstration of resistance may only be a reflexion of the technique employed for testing.

We are grateful to D. W. Richards and the staff of the laboratory for technical assistance. H. Hardie, M.R.C.V.S., of Abbot Laboratories is to be thanked for the supply of spectinomycin disks and Mrs J. M. Thresher for typing the manuscript.

REFERENCES

- GARROD, L. P. & O'GRADY, F. (1971). *Antibiotics and Chemotherapy*, 3rd ed. Edinburgh: E. & S. Livingstone.
- HINTON, M. (1974). *Salmonella dublin* abortion in cattle: studies on the clinical aspects of the condition. *British Veterinary Journal* **130**, 536.
- SOJKA, W. J. & HUDSON, E. B. (1976). A survey of drug resistance in *Salmonella* isolated from animals in England and Wales during 1972. *British Veterinary Journal* **132**, 95.
- SOJKA, W. J., HUDSON, E. B. & SLAVIN, G. (1974). A survey of drug resistance in *Salmonella* isolated from animals in England and Wales during 1971. *British Veterinary Journal* **130**, 128.
- WHITE, G. & WEST, B. (1974). Sulphonamide testing of *Salmonella dublin*. *Veterinary Record* **95**, 467.