Salmonella serotypes, Salmonella typhi phage types, and anti-microbial resistance at the University Hospital of the West Indies, Jamaica

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SUMMARY

Bacteriologically proved cases of salmonellosis presenting at the University Hospital have increased nearly threefold since 1957. The most striking change has been a considerable increase in the incidence of *Salmonella heidelberg* and *Salmonella derby* in the last 5 years, probably resulting from hospital acquired infection.

About 80 cases of typhoid fever are reported each year in Jamaica. There has been little change in the prevalence of different phage types of *Salmonella typhi* since 1961. Paratyphoid fever is rare.

Standardized antimicrobial disk-sensitivity testing was performed on selected surviving salmonella strains since 1964. S. typhi has remained fully sensitive to all the agents tested except streptomycin. Other salmonellas, however have shown a significant increase in antimicrobial resistance since 1970. Most of this increase was due to multiple resistance in S. heidelberg and S. derby, and the survival and dissemination of these strains in the hospital environment may be related to antibiotic usage.

INTRODUCTION

In recent years studies from the Department of Microbiology at the University of the West Indies have revealed a high and apparently increasing degree of antimicrobial resistance in bacterial pathogens isolated at the University Hospital. (James & Grant, 1972; French, King & Ramachander 1976). Unfortunately it is not always possible to compare present antibiotic sensitivities with past reports because the methods of sensitivity testing have varied, and in the past have not been properly controlled.

However, most strains of *Salmonella* from this hospital have been stored on agar slopes and are thus available for antibiotic sensitivity testing with standardized methods. It was decided, therefore, to compare the antimicrobial sensitivities of recently isolated salmonella strains with those isolated in previous years.

This paper reports standardized antimicrobial disk-sensitivity testing of surviving salmonella strains isolated at this hospital (and *Salmonella typhi* from the whole island) during selected years from 1964 to 1975. We also discuss the prevalence of salmonella serotypes and *S. typhi* phage types since 1957, and the relation between antimicrobial resistance and the epidemiology of salmonella infection seen at this hospital.

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MATERIALS AND METHODS

Organisms

Strains of *S. typhi* were isolated from patients at the University Hospital of the West Indies (UHWI) or were submitted from other parts of the island. All other salmonellas were isolated from clinical cases presenting at the University Hospital. Each strain was isolated from a different patient and stored at room temperature on Dorset egg slopes.

Organisms were isolated and identified, and salmonella serotyping was performed, by the methods of Edwards & Ewing (1972). S. typhi phage typing was performed by the method of Anderson & Williams (1956).

Antibiotic sensitivity testing

All surviving strains of S. typhi from the years 1965, 1969, 1970, 1974 and 1975, and all surviving strains of other salmonellas from the years 1964, 1965, 1969, 1970 and 1975 were tested for antibiotic sensitivity by the disk method of Stokes & Waterworth (1972).

The medium used was Oxoid DST agar with 5 % lysed horse blood. The inoculum was one drop of an overnight broth culture spread with a dry swab. After overnight incubation at 37° C this produced a dense but not confluent growth. Oxoid Multodisks were used, and the antibiotics tested and their concentration per disk were as follows: ampicillin 10 μ g, chloramphenicol 10 μ g, tetracycline 10 μ g, streptomycin 10 μ g, sulphatriad (sulphonamide) 300 μ g, cotrimoxazole 25 μ g.

The diameters of the zones of inhibition were measured with callipers, taking the edge of the zone as the point of complete inhibition of growth. The zone sizes in millimetres were compared with those of a standard sensitive control organism (the Oxford Staphylococcus, NCTC 6571) and the results expressed as 'sensitive' 'moderately sensitive' or 'resistant' according to the criteria of Stokes & Waterworth. 'Moderately sensitive' strains are considered to be clinically responsive to increased antibiotic dosage and are included in the designation 'sensitive' in the following results. When an organism was found to be resistant to three or more of the antibiotics tested, the organism is referred to as being multiply resistant.

Statistical methods

Differences between the percentage of resistant strains in each group were analysed by standard methods (Bradford Hill, 1971). Differences which exceed twice the standard error are designated 'significant'.

RESULTS

Distribution of salmonella serotypes

Salmonella strains isolated in Jamaica were first serotyped in 1952 (Grant & Caselitz, 1954b). Since then a series of papers have reported the results of salmonella serotyping and *S. typhi* phage typing in the island (Grant & Caselitz, 1954*a*, *b*; Grant, Gracey & Clark, 1957; King & Grant, 1963; Bisno & Grant, 1968). This paper brings this information up to date and summarizes the results since 1957.

Table 1 shows the prevalence of various serotypes of salmonellas isolated from cases presenting at this hospital from 1962 to 1975. The total number of cases has varied from a low of 26 in 1964 to highs of 164 in 1973 and 125 in 1975.

Table 2 summarizes the prevalence of the commonest serotypes since 1957, grouped in 5 year periods. The total number of salmonella infections has shown a general increase: 163 cases were seen in each of the 5 year periods 1957–61 and 1961–5, 291 during 1966–70 and 468 during 1971–5. The number of different serotypes identified has increased since 1952 (Table 1), although most serotypes are responsible for only one or two cases of infection in each year. Altogether 78 different serotypes have been identified from human cases of salmonellosis at this hospital since 1952, but only 46 of these were seen in the last 5 years. Table 2 gives the prevalence of the ten most important serotypes are constantly responsible for about 30 % of cases.

S. typhimurium has been the commonest serotype, which is similar to the picture in most other areas of the world. However, although the number of cases of S. typhimurium infection has remained relatively constant, the proportion of cases compared with other serotypes has fallen.

S. infantis, S. saintpaul and S. oranienburg have remained fairly common causes of infection, while S. anatum has become less common, and S. choleraesuis has not been seen since 1965. S. enteritidis has appeared more frequently since 1969 and was responsible for 11 cases in 1975. S. ohio was first seen in 1972 and caused 12 cases in 1975. However, the most striking increase has been in the prevalence of S. heidelberg and S. derby.

S. heidelberg was not seen in this hospital before 1966, and during the seven years 1966–72 it was responsible for 14 cases. In 1973 there was an outbreak of 39 cases, and a further 15 were seen in 1974 and 14 in 1975. S. derby has usually been responsible for a few cases each year at this hospital and the numbers have been increasing as the overall incidence of salmonellosis has increased (Table 2). However, the total of 50 cases for the period 1971–5 was largely due to 31 cases seen during 1975 alone.

Six cases of S. paratyphi A were seen in Jamaica between 1952 and 1956 (Grant et al. 1957). No further cases have been identified at the UHWI since then. S. paratyphi C accounted for 2 cases between 1952–6 and 1 case between 1957–61, but no further cases have been seen. S. paratyphi B was first seen in 1968, and only 3 cases have been identified up to 1975 (Table 1). Paratyphoid infection is of small importance in Jamaica compared with typhoid fever, and, furthermore, some of these paratyphoid cases have occurred in visitors to the island who probably contracted their infection abroad.

Distribution of S. typhi phage types

The number of strains of S. typhi submitted for phage typing is only a small proportion of the number of cases notified to the department of health. Table 3 shows the prevalence of the various phage types among those cultures which were tested and the number of notified cases of typhoid fever since 1961. In the last 10 years there have been about 80 cases of typhoid fever notified per annum. The

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	Serotype	S. havana	S. tunis	$S.\ worthington$	S. chameleon	S. aluchua	S. bere	S. bonaire	S. cerro	$S.\ marina$	S. ruiru	S. siegberg	Untyped	Total
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Serotype	1957-1961*	1961-1965	1966-1970	1971-1975
S. typhimurium	57 (35)†	44 (27)	60 (21)	60 (13)
S. heidelberg	(0)	(0)	9 (3)	73 (16)
S. derby	13 (8)	10 (6)	27 (9)	50 (11)
S. infantis	13 (8)	23 (14)	32 (11)	35 (7)
S. saintpaul	13 (8)	13 (8)	8 (3)	34 (7)
S. oranienburg	1 (1)	9 (6)	16 (5)	26 (6)
S. enteritidis	7 (4)	2 (1)	5 (2)	31 (7)
S. ohio	(0)	(0)	(0)	20 (4)
S. anatum	12 (7)	9 (6)	4 (1)	3 (1)
S. choleraesuis	12 (7)	2 (1)	(0)	(0)
Others	35 (22)	51 (31)	130 (45)	136 (29)
Total salmonella	163	163	291	468

Table 2. Salmonella cases presenting at the University Hospital of the West Indies. The ten most important serotypes (excluding S. typhi)

* From King & Grant (1963).

† The figures in parentheses are percentages.

Table 3. Salmonella typhi pha	ge types identified in Jamaica,
and cases of typhoid fer	ver notified, 1957–1975

Phage type	1957-1961*	1961-1965*	1966-1970	19711975
Α	37 (4.9)†	25 (3 ·0)	4 (2·3)	12 (7.5)
C ₁	4 (0.5)	3 (0.4)	<u> </u>	·
C4	37 (4.9)	21 (2.5)	7 (4.0)	11 (6.8)
$\mathbf{E_1}$	481 (63.6)	609 (73·0)	112 (63.6)	103 (64.0)
\mathbf{E}_{9}	_	7 (0.8)		
M	1 (0.1)			
т	2 (0.3)	_		
38		1 (0.1)		
45	104 (13.8)	102 (12.2)	3 5 (19·9)	22 (13.7)
Vi negative	26 (3·4)	38 (4.6)	10 (5.7)	3 (1.9)
Vi degraded	64 (8.5)	28 (3.4)	8 (4.5)	10 (6.2)
Total typed	756	834	176	161
Cases notified	Not available	922	406	408

* Figures from King & Grant (1963), Bisno & Grant (1968).

† Figures in parentheses are percentages.

commonest phage type is E_1 , which accounts for about 60% of Jamaican strains, and this is also the commonest phage type worldwide. Type 45 accounts for about 15% and types A and C4 for about 5% each. About 10% of the cultures tested have been untypable: about 5% Vi degraded and 5% Vi negative (Table 3). There has been little change in the proportions of these phage types since 1957. Although a few cases of types C1, E9, M, T and 38 were seen in earlier years, none of these types have been isolated since 1965. Type 45 is almost unique to Jamaica (Report, 1973).

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 Table 4. Antimicrobial resistance of surviving strains of salmonellas (excluding S. typhi)

	Number of		Pe	ercentage	of strains	resistant	to	
	strains tested	Ampi- cillin	Chloram- phenicol			Tetra- cycline	Strepto- mycin	Three or more
1964-5	53	$5 \cdot 6$	5.6	0	1.9	9·4	15.1	0
1969-70	92	9.7	3 ·2	0	$3 \cdot 2$	$4 \cdot 3$	47.8*	$2 \cdot 2$
1975	102	41 ·1*	35.3*	7.8*	39 ·2*	14.7*	47.1	37.3*

* Significant difference from earlier strains, see text.

Table 5. Antimicrobial resistance of surviving strains of salmonellas (excluding S. typhi) isolated during 1975

	Number		Pe	ercentage	of strains	resistant	to	
	of strains tested	Ampi- cillin	Chloram- phenicol		Sulphon- amide	Tetra- cycline	Strepto- mycin	Three or more
S. derby and S. heidelberg	4 0	82·5*	75.0*	20 ∙0 *	77.5*	35.0*	80.0*	80.0
Other salmonellas	62	1 4 ·5	7.7	0	14.5*	1.6	25.8*	7.7
\mathbf{Total}	102	41 ·1*	35.3*	7.8*	39 ·2*	14.7*	47.1	37.3*
	* Si	gnificant	difference	s from ea	rlier strain	ls, see tex	t.	

Antimicrobial resistance of salmonellas other than S. typhi

Fifty-three strains of *Salmonella* were available for testing from the period 1964-5, 92 from 1969-70, and 102 from 1975. Table 4 shows that since 1964 there has been a striking increase in resistance and multiple resistance to all the drugs tested.

The prevalence of streptomycin resistance rose from $15 \cdot 1\%$ in 1964-5 to $47 \cdot 8\%$ in 1969-70. There was no further increase in resistance to this antibiotic in 1975. However, for all the other drugs tested, the percentage of resistant strains and the prevalence of multiple resistance increased significantly in 1975 compared with 1964-5 and 1969-70.

Of the 1975 strains, $37\cdot3\%$ showed multiple resistance, $41\cdot1\%$ resistance to ampicillin, $35\cdot3\%$ to chloramphenicol and $39\cdot2\%$ to sulphonamide: $14\cdot7\%$ showed resistance to tetracycline and $7\cdot8\%$ resistance to cotrimoxazole. (Table 4).

Two serotypes – S. heidelberg and S. derby – accounted for 40 of the 102 strains tested from 1975. These two types together were responsible for most of the increase in antibiotic resistance seen in 1975 (Table 5). S. heidelberg was usually resistant to all the drugs tested, and was responsible for all the cotrimoxazole resistance. S. derby was usually sensitive to tetracycline and cotrimoxazole, but resistant to ampicillin, chloramphenicol, streptomycin and sulphonamide. If S. heidelberg and S. derby are excluded from the 1975 results, the remaining strains show no significant increase in antibiotic resistance compared with 1969–70, except for sul-

	Number		Pe	ercentage	of strains	resistant	to	
	strains tested	Ampi- cillin			Sulphon- amide		Strepto- mycin	Three or more
1965	44	4.5	0	0	0	2.3	4 ·5	0
1969-70	19	0	0	0	0	6·3	94 ·7*	0
1974-5	21	0	0	0	0	0	100	0

Table 6. Antimicrobial resistance of surviving strains of S. typhi

* Significant difference from earlier strains, see text.

phonamide (Table 5). These strains also show a significant but unexplained decrease in the incidence of resistance to streptomycin.

Antimicrobial resistance of S. typhi

Only 44 strains of S. typhi were available for testing from 1965, 19 from 1969–70, and 21 from 1974–5. Table 6 shows that S. typhi has remained fully sensitive to all the drugs tested except streptomycin. As with the other salmonellas, streptomycin resistance had become fully established by 1970. The small changes in ampicillin and tetracycline resistance are not significant. All strains of S. typhi remained fully sensitive to chloramphenicol.

DISCUSSION

This paper is mainly concerned with bacteriologically proved cases of salmonella infection seen at the University of the West Indies. It is therefore not necessarily representative of salmonellosis in Jamaica as a whole, and is biased in favour of hospital-acquired salmonellosis and severe infection in children.

Our results for antimicrobial sensitivity testing may tend to underestimate the degree of antimicrobial resistance in the earlier isolates, since bacteria may spontaneously lose transmissible resistance factors during storage. However, the general sensitivity of strains in 1965 and 1970 accords with clinical impressions from that period, and the results for the 1975 strains are similar to those previously found on routine testing (French *et al.* 1976).

While S. typhi has remained fully sensitive to all the drugs tested except streptomycin, resistance and multiple resistance has increased greatly in the other salmonellas, especially in the last 5 years. S. typhi is nearly always communityacquired, but the majority of the other salmonella strains were from hospitalized cases. These results, with standardized sensitivity tests for stored salmonella strains, corroborate the clinical impression that resistance and multiple resistance has become increasingly common amongst gram-negative organisms isolated at this hospital in recent years.

The high degree of resistance amongst salmonella strains isolated during 1975 has been mainly due to large numbers of cases of infection with resistant S. *heidelberg* and S. *derby*, which together caused 35% of all cases in that year. S. *heidelberg* has been responsible recently for many cases of nosocomial infection at

this hospital, especially in children (in preparation), and the same is probably true for S. derby. Both these organisms showed a high degree of multiple resistance, and this factor undoubtedly favours their survival and dissemination in the hospital environment.

It is probable that increasing antibiotic resistance, and especially increasing multiple resistance, is often initially due to the dissemination of a single resistant type. Routine typing of oganisms such as *Escherichia coli* or *Klebsiella* is not normally performed, so that for these organisms the appearance of a single resistant type is usually perceived as a general increase in resistance amongst the species concerned. When typing is carried out – usually during a hospital outbreak of resistant infection – the resistant organisms are often found to belong to the same type and to have a common source. Farrant & Tomlinson (1966) have observed this phenomenon in *Shigella*. They studied the epidemiology of Sonne dysentery in London over a 10-year period, and observed that the antibiotic resistance seen in shigella strains merely reflected the resistance pattern of the dominant epidemic strain. This present study shows a similar effect: resistant serotypes have become the common causes of salmonellosis and have thus produced a high proportion of antimicrobial resistance, even though serotypes other than *S. heidelberg* and *S. derby* have generally remained sensitive.

A recent survey of salmonellosis in the United States from 1963–74 (Ryder, Merson, Pollard & Gangarosa 1976) shows that the commonest reported serotype in both humans and animals is still S. typhimurium. In human cases S. enteritidis and S. newport accounted for 7.8% and 7.2% of cases respectively, and S. heidelberg, S. infantis and S. saintpaul for about 5% each. S. derby was responsible for only 2.1% of human cases, and was the ninth commonest serotype. Thirty-three per cent of the human cases were caused by uncommon serotypes, each of which accounted for less than 2% of the the total – results similar to our findings at the UHWI. S. heidelberg was responsible for 10.7% of the non-human strains in the U.S. from 1968–74, second only to S. typhimurium, and S. derby was the ninth most common serotype isolated from animals, accounting for 4.6%.

Lee (1974) has recently reviewed salmonellosis in England and Wales. S. typhimurium is again the most common serotype, but the considerable increase in incidence of salmonellosis in England and Wales from 1966 to 1971 was mainly due to an increase in the isolations of S. enteritidis, S. panama, S. stanley, S. virchow, S. agona, S.4, 12:d:— and S. indiana. Most of these serotypes have pig and poultry reservoirs. S. heidelberg was relatively unimportant in England and Wales up to 1950, but from 1956-65 it was the second most common cause of salmonella food poisoning (Wilson & Miles, 1975). Since 1966 it has dropped to sixth place. S. derby is an uncommon cause of salmonellosis in Britain.

These reviews confirm previous observations (Bisno & Grant, 1968) that the distribution of the salmonella serotypes in Jamaica resembles that in the United States rather than that in Britain. The unusually high prevalence of S. heidelberg and S. derby reported here probably results from the clustering of hospital-acquired infection.

It is likely that the multiple resistance seen in salmonellas isolated at the UHWI

Table 7. Number of grams of ampicillin and chloramphenicol dispensed by the hospital pharmacy, University Hospital of the West Indies, 1969–1975

	1969	1970	1971	1972	1973	1974	1975
Ampicillin	11863	17213	13064	22194	33833	34735	32578
Chloramphenicol	NA	NA	11529	10248	16059	11534	14308
		NA; f	igures not	available.			

is transmissible. James, Wells & Grant, (1973) have shown that transmissible resistance does occur in other gram-negative organisms isolated at this hospital, and transmissible resistance in salmonellas is common elsewhere (Neu *et al.* 1975; Anderson, 1974).

The resistance pattern seen in Jamaican strains of S. derby (resistant to all except tetracycline and cotrimoxazole) has recently appeared in some strains of S. ohio. This latter serotype has also become increasingly common in Jamaica in the last few years (Tables 1 and 2) and may have gained its present resistance pattern by transmission of a plasmid from S. derby.

Transmissible resistance to chloramphenicol and other drugs has already appeared in S. typhi in Mexico, Vietnam and elsewhere (Anderson, 1975), and there is a serious danger that such multiple resistance may appear in endemic strains of S. typhi in Jamaica and leave us with no effective treatment for typhoid fever. The resistance of Jamaican strains of S. heidelberg to chloramphenicol, ampicillin, and cotrimoxazole is especially worrying, since these three drugs are the ones of choice for typhoid fever.

James and his co-workers have demonstrated that a major factor contributing to the emergence of transferable resistance in Jamaican strains of enterobacteria has been the increasing use of antibiotics, both in hospital and as animal feed supplements. (James *et al.* 1975; Wells & James, 1973). Table 7 shows the number of grams of ampicillin and chloramphenicol dispensed at the University Hospital of the West Indies since 1969. The use of ampicillin has increased nearly threefold since 1969, and the use of chloramphenicol remains at an unreasonably high level. These two drugs are now relatively ineffective in the treatment of gram-negative infection at this hospital (James *et al.* 1973; French *et al.* 1976), and can usually be replaced by other more effective agents such as cotrimoxazole.

It is possible that a reduction in the use of ampicillin and chloramphenicol at this hospital over the next 5 years might restore the sensitivities of salmonellas and other gram-negative organisms thereby ensuring the continuing sensitivity of endemic strains of S. typhi. In view of the present high degree of antimicrobial resistance in Jamaican salmonella strains and the increasing prevalence of salmonellosis at this hospital, such a policy of ampicillin and chloramphenicol restriction would seem to be urgently required.

We are grateful to the staff of the enteric laboratory who over the years have performed the isolation and typing of the organisms described in this paper, and to Mr L. Forbes, Chief Hospital Pharmacist, who provided the information for Table 7.

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