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Resistance to chloramphenicol and ampicillin in *Salmonella johannesburg* in Hong Kong: observations over a five-year period 1973–1977

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SUMMARY

Salmonella johannesburg has been prevalent in Hong Kong since 1973 and most strains were resistant to a multiplicity of antibiotics. The susceptibility of *S. johannesburg* strains isolated in a 5-year period from 1973 to 1977 to eight antimicrobial drugs including ampicillin (A), streptomycin (S), tetracycline (T), chloramphenicol (C), Kanamycin (K), sulphadiazine (Su), trimethoprim (Tm) and gentamicin (G) was tested by the agar dilution method. The proportion of strains resistant to chloramphenicol and ampicillin increased steadily during the 5-year period while those resistant to tetracycline decreased dramatically. This change was associated with an alteration of predominant patterns of antibiotic resistance: strains with the resistance pattern A.S.T.C.K.Su predominated in years 1973, 1974 and 1975 while those with the resistance pattern A.S.C.K.Su predominated in years 1976 and 1977. Analysis of the resistance patterns of *S. johannesburg* strains isolated from the same cases showed that the resistance pattern A.S.C.K.Su was more stable, and changed less frequently to other patterns of resistance than strains with the resistance pattern A.S.T.C.K.Su. In donor salmonella strains with the A.S.T.C.K.Su resistance pattern, transmissible factors carrying resistance to A.S.T.C.K.Su *in toto*, to A.S.T.C.Su, A.T.C.K.Su and to A or T alone were demonstrated. In donor salmonella strains with the A.S.C.K.Su resistance pattern, transmissible factors carrying resistance to A.S.C.K.Su *in toto* and to A.S.K.Su were detected. The significance of the carriage of such transmissible resistance factors by this *Salmonella* is briefly discussed.

INTRODUCTION

Resistance to chloramphenicol among salmonellas was extremely rare in the past. It became of clinical concern after the occurrence of an epidemic of typhoid fever due to a *Salmonella typhi* carrying chloramphenicol-resistant factors in Mexico in 1972 (Anderson, 1975). Resistance to chloramphenicol among other salmonellas isolated from clinical cases has also been increasingly reported thereafter (McHugh *et al.* 1975; Grant, Bannatyne & Shapley, 1976; Cherubin *et al.* 1977). The occurrence of such chloramphenicol-resistant Salmonella strains however remains relatively infrequent in most countries. This was believed to be

due to the fact that chloramphenicol resistance in *Salmonella* was relatively unstable. The resistance factors are readily lost *in vivo* and *in vitro* in the absence of selection pressure (Cherubin *et al.* 1977). In Hong Kong, chloramphenicol-resistant salmonella strains were mainly found in *S. johannesburg* which has been the most prevalent salmonella serotype isolated in this locality since 1973 (Teoh-Chan *et al.* 1977). Many strains of this *Salmonella* were resistant to a multiplicity of antibiotics including chloramphenicol and ampicillin. Thus, we had an opportunity to study the sequential changes of chloramphenicol and ampicillin resistance in a given salmonella serotype in a confined locality within a 5-year period from 1973 to 1977.

MATERIALS AND METHODS

Bacterial strains

Three hundred and eighty-two strains of *Salmonella johannesburg* isolated from clinical specimens in a general hospital in Hong Kong during a 5-year period from January 1973 to December 1977 were tested. Of these, 376 strains were isolated from faecal specimens from children with diarrhoea, four from pus specimens, one from blood and one from urine. Chloramphenicol was rarely if ever given to the patients for antibiotic therapy during their stay in the hospital. These strains were kept on agar slopes before testing for antibiotic susceptibility as described in this study.

In conjugation experiments with the chloramphenicol-resistant *S. johannesburg* strains as the donor, two recipient *Escherchia coli* K12 strains were used. One was a nalidixic acid resistant mutant UB1139 (Lac$^+$, *leu*, *met*, *thy*, F$^-$, λ^-, R$^-$, Nx^R) kindly provided by Professor M. H. Richmond, Department of Bacteriology, University of Bristol, England; the other was a rifampicin resistant mutant JP995 (Lac$^+$, prototroph, F$^-$, λ^-, R$^-$, Rif^R) kindly supplied by Professor A. J. Pittard, Department of Microbiology, University of Melbourne, Australia.

Antibiotic susceptibility test

The susceptibility of the salmonella strains to various antibiotics (the term 'antibiotics' here includes chemotherapeutic agents for convenience) was tested by the agar dilution method as described by Barry (1976). The antibiotics and their concentrations tested were as follows: ampicillin (A), 25 μg/ml; streptomycin (S), 15 μg/ml; tetracycline (T); 10 μg/ml; chloramphenicol (C), 25 μg/ml; kanamycin (K), 10 μg/ml; sulphadiazine (Su), 100 μg/ml; trimethoprim (Tm), 2 μg/ml and gentamicin (G), 8 μg/ml. Susceptibility tests for trimethoprim and sulphadiazine were performed on Sulphonamide Antagonist Free Medium (SAF, Mast, Liverpool, England) while those for other antibiotics on Diagnostic Sensitivity Test Medium (DST, Oxoid, England). In addition, the minimal inhibitory concentrations (MICs) of chloramphenicol were determined for all the chloramphenicol-resistant strains.

Conjugation procedure

For testing transfer of resistance, cultures of donor and recipient bacteria grown at 37 °C to late-logarithmic phase in nutrient broth were mixed at a proportion of 40 μl of donor and 200 μl of recipient bacteria in 3 ml of broth. After an initial mixing for 1 min on a Vortex Mixer, the 'mating broth' was incubated at 37 °C overnight and 0·1 ml volumes of the 'mating broth' were plated onto selective MacConkey medium after appropriate dilution. Selection was made with chloramphenicol (25 μg/ml), tetracycline (10 μg/ml), ampicillin (25 μg/ml), streptomycin (15 μg/ml) and kanamycin (10 μg/ml) and counter-selection of the donor strains with nalidixic acid (25 μg/ml) or rifampcin (25 μg/ml). The frequencies for transfer of resistance were calculated as the number of the transconjugant c.f.u. of a particular recombinant type divided by the total number of the donor c.f.u. In case the transfer frequency was very low, the mixture of donor and recipient bacterial cells in the 'mating broth' was passed immediately after mixing, through a sterile 25 mm Millipore filter with a pore size of 0·45 μm (Millipore Corp., New Bedford, Mass. U.S.A.). The filter containing the bacteria was placed on a brain heart infusion agar plate and incubated at 37 °C overnight. Bacterial cells grown on the filter were resuspended in broth and after appropriate dilution plated on selective MacConkey agar.

RESULTS

Antibiotic susceptibility

Resistance of the 382 *Salmonella johannesburg* strains to individual antibiotics is given in Table 1. Only three out of these strains were sensitive to all the eight antibiotics tested. Most of the strains were resistant to ampicillin, streptomycin, tetracycline, chloramphenicol, kanamycin and sulphadiazine. Six strains were resistant to trimethoprim but none to gentamicin. It was interesting to note that while the proportion of strains resistant to chloramphenicol and ampicillin, as well as to streptomycin, kanamycin and sulphadiazine increased steadily during the 5-year period from 1973 to 1977, there was a steep drop in the proportion of tetracycline-resistant strains over the same period.

Most strains were found resistant to a multiplicity of antibiotics; 337 out of the 382 strains tested were resistant to three or more. The patterns of resistance most frequently encountered are given in Table 2. Two patterns of resistance predominated. One was a concomitant resistance to six drugs, A.S.T.C.K. and Su, and the other was a resistance to the same group without tetracycline. In addition to the above two resistance patterns, there were eight other patterns of chloramphenicol resistance. These patterns together with the MICs of chloramphenicol are shown in Table 3. The MICs of chloramphenicol for most strains with the resistance pattern A.S.C.K.Su were 320 μg/ml while those for most strains with the resistance pattern A.S.T.C.K.Su varied from 320 to 640 μg/ml. The sharp decrease in the proportion of tetracycline-resistant *S. johannesburg* strains as indicated in Table 1 was apparently associated with the drastic change of the predominant resistance pattern from A.S.T.C.K.Su to A.S.C.K.Su. Such

Table 1. *Resistance to eight anti-bacterial drugs of* S. johannesburg *isolated during a 5-year period from* 1973 *to* 1977

Resistance to	1973	1974	1975	1976	1977	Total
Ampicillin	44 (70)*	44 (86)	109 (88)	116 (98)	26 (100)	339 (89)
Streptomycin	45 (71)	31 (61)	97 (78)	115 (98)	26 (100)	314 (82)
Tetracycline	57 (90)	48 (94)	95 (77)	8 (7)	0	208 (54)
Chloramphenicol	29 (46)	31 (61)	86 (69)	113 (96)	26 (100)	285 (75)
Kanamycin	42 (67)	43 (84)	99 (80)	112 (95)	26 (100)	322 (84)
Sulphadiazine	60 (95)	42 (82)	113 (91)	116 (98)	26 (100)	357 (93)
Trimethoprim	0	0	5 (4)	1 (1)	0	6 (2)
Gentamicin	0	0	0	0	0	0
Total no. of strains	63	51	124	118	26	382

* Figures in parentheses indicate percentages to the total number of strains isolated in that particular year.

Table 2. *Some frequent patterns of antibiotic resistance in* S. johannesburg *isolated over a* 5-year *period from* 1973 *to* 1977

Patterns of resistance*	1973	1974	1975	1976	1977	Total
A.S.T.C.K.Su	28 (44)†	27 (53)	59 (48)	5 (4)	—	119 (31)
A.S.C.K.Su	—	—	17 (14)	105 (90)	26 (100)	148 (39)
A.S.T.K.Su	12 (19)	3 (6)	6 (5)	—	—	21 (5)
A.S.T.C.K.Su.Tm	—	—	4 (3)	—	—	4 (1)
A.T.K.Su	—	6 (12)	4 (3)	—	—	10 (3)
T.Su	14 (22)	3 (6)	4 (3)	1 (1)	—	22 (6)
Other patterns	9 (14)	12 (24)	30 (24)	7 (6)	—	58 (15)
Total no. of strains	63	51	124	118	26	382

* A, Ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; K, kanamycin; Su, sulphadiazine; Tm, trimethoprim.

† Figures in parentheses indicate percentages to the total number of strains isolated in that particular year.

a change of patterns occurred over a period of 3 years between 1975 and 1977. There was a concurrent diminution in the varieties of other patterns of resistance, such that all the *S. johannesburg* strains isolated in 1977 showed only one resistance patterns, A.S.C.K.Su (Table 4).

Patient studies

In many cases, it was possible to isolate *S. johannesburg* repeatedly from faecal specimens from the same patient three or more times over a period. Thus, the change of patterns of antibiotic resistance could be followed up. Changes of resistance patterns related to the two major patterns of chloramphenicol resistance, A.S.T.C.K.Su and A.S.C.K.Su, are shown in Table 5. It appeared that the resistance pattern A.S.T.C.K.Su was relatively unstable, and changed more frequently to other patterns of resistance. By contrast, the resistance pattern

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Table 3. *The minimal inhibitory concentrations of chloramphenicol for the chloramphenicol-resistant* S. johannesburg *strains*

Patterns of chloramphenicol resistance*	Total no. of strains	No. of strains with MICs (μg/ml) of chloramphenicol of			
		160	320	640	1280
A.S.T.C.K.Su	119	10	50	56	3
A.S.T.C.K.Su.Tm	4	—	—	2	2
A.S.C.K.Su	148	2	116	30	—
A.S.C.K.Su.Tm	1	—	1	—	—
A.S.T.C.Su	4	—	3	1	—
A.S.C.Su	2	—	1	1	—
A.T.C.Su	2	—	2	—	—
A.T.C.K.	2	—	1	1	—
T.C.Su	2	1	—	1	—
T.C.	1	—	—	1	—
Total number of chloramphenicol resistant strains	285	13	174	93	5

* Abbreviations as in Table 2.

Table 4. *Changes in the number of resistance patterns revealed in relation to the number of* S. johannesburg *strains examined over a 5-year period from* 1973 *to* 1977

	1973	1974	1975	1976	1977
No. of strains examined	63	51	124	118	26
No. of resistance patterns revealed	9	11	20	9	1
Ratio of the number of strains examined to the number of patterns revealed	7:1	5:1	6:1	13:1	26:1

Table 5. *Change of antibiotic resistance patterns in* S. johannesburg *isolated from the same patients**

Resistance patterns†	Change of patterns	Patients with *S. johannesburg* isolates showing such changes	
		No. of patients	(%)
A.S.T.C.K.Su	Unchanged	10	40
	Changed from other patterns	8	32
	Changed to other patterns	7	28
Total		25	100
A.S.C.K.Su	Unchanged	18	81
	Changed from other patterns	1	5
	Changed to other patterns	3	14
Total		22	100

* Only patients with three or more isolates of *S. johannesburg* obtained over a period of more than 7 days were included in this table.

† Abbreviations as in Table 2.

Table 6. *Change of antibiotic resistance patterns of* S. johannesburg *strains isolated from patient*

Date of isolation (1975)	Pattern of resistance	Date of isolation	Pattern of resistance
17.vi.	A.S.T.C.K.Su	5.viii.	A.S.T.C.K.Su
27.vi.	A.S.T.C.K.Su	19.viii.	A.S.T.C.K.Su
2.vii.	A.S.T.C.K.Su	1.ix.	A.S.K.Su
11.vii.	A.S.T.C.Su	17.x.	A.S.K.Su
18.vii.	A.S.T.C.K.Su	19.xi.	A.S.K.Su
22.vii.	T		
29.vii.	T		

A.S.C.K.Su was relatively stable and remained unchanged in the majority of patients. The *in vivo* instability of the resistance pattern A.S.T.C.K.Su can be illustrated by the following two examples.

Case 1. An 8-day-old male baby with diarrhoea. *S. johannesburg* was isolated from his faeces on 29 xii 1973 and on 8 i 1974. Both isolations showed a resistance pattern of A.S.T.C.K.Su. He was discharged and 2 months later re-admitted to the hospital because of chronic diarrhoea. *S. johannesburg* was again isolated but was found sensitive to all the above six antibiotics to which it was originally resistant.

Case 2. A 3-month-old girl, with Down's syndrome, congenital heart disease and chronic diarrhoea. *S. johannesburg* was repeatedly isolated from her stool specimens. The first three isolations were resistant to A.S.T.C.K. and Su, while the subsequent ones showed change of resistance patterns as shown in Table 6.

Transfer of resistance

The results of transfer of resistance from some representative *S. johannesburg* strains to *Escherichia coli* UB1139 and JP995 recipient strains by conjugation experiments are shown in Table 7. From all the four donor salmonella strains showing the A.S.T.C.K.Su resistance pattern, resistance to A.S.T.C.K.Su *in toto* was able to be co-transferred successfully to JP995, irrespective of whether the selection was made by ampicillin, streptomycin, chloramphenicol, tetracycline or kanamycin alone. Thus, resistance to A.S.T.C.K and Su appeared to be carried either by a single genetic unit or units that were closely linked together. The frequencies of such a transferrence for resistance at 37 °C in overnight mating ranged from 3×10^{-3} to 2×10^{-5}, depending on the donor strains concerned and the antibiotics used for selection. On the other hand, though resistance to A.T.C.K.Su was able to be co-transferred to UB1139, there was no transfer of resistance to streptomycin to this recipient strain. It was quite possible that resistance to streptomycin was transferred to but not expressed by UB1139. The MIC of chloramphenicol transferred to *E. coli* (UB1139) recipient was found to be 80 μg/ml and that of tetracycline 100 μg/ml, while the MICs of chloramphenicol and tetrachcyline of the donor salmonella strains were 320 and 400 μg/ml,

Table 7. *Resistance patterns transferred by some* S. johannesburg *strains to* E. coli *UB*1139 *and JP*995 *recipients*

Antibiotic resistance patterns of the donor strain*	Antibiotics used for selection	Transferred resistance pattern (no. of donor strains transferring resistance shown in parentheses)	
		In UB1139	In JP995
A.S.T.C.K.Su (four strains)	Chloramphenicol	A.T.C.K.Su† (4); A.T.C.Su (1)	A.S.T.C.K.Su (4); A.S.T.C.Su (1)
	Tetracycline	A.T.C.K.Su (4); T (2)	A.S.T.C.K.Su (4); T (2)
	Ampicillin	A.T.C.K.Su (4); A (4)	A.S.T.C.K.Su (4); A (1)
	Kanamycin	A.T.C.K.Su (4)	A.S.T.C.K.Su (4); A.T.C.K.Su (1)
	Streptomycin	Not detected	A.S.T.C.K.Su (4); A.S.T.C.Su (1)
A.S.C.K.Su (one strain)	Chloramphenicol	A.C.K.Su (1)	A.S.C.K.Su (1)
	Ampicillin	A.C.K.Su (1)	A.S.C.K.Su (1); A.S.C.Su (1)
	Kanamycin	A.C.K.Su (1)	A.S.C.K.Su (1)
	Streptomycin	A.C.K.Su (1); A.K.Su (1)	A.S.C.K.Su (1)
A.T.C.Su (1 strain)	Chloramphenicol	T.C. (1)	A.T.C.Su (1); T.C. (1)
	Tetracycline	T.C. (1)	A.T.C.Su (1); T.C. (1)
T.C.Su (1 strain)	Chloramphenicol	T.C. (1)	T.C. (1)
	Tetracycline	T.C (1); T (1)	T.C.Su (1); T.C. (1)

* Abbreviations as in Table 2.

† In case UB1139 was used as the recipient, resistance to sulphonamide was tested on MacConkey agar medium containing 100 μg/ml of sulphadiazine, a sulphonamide sensitive *E. coli* strain (NCTC10418) as well as a resistant one were used as controls, as sulphonamide sensitive *E. coli* strains grew less luxuriantly than the resistant *E. coli* strains and usually did not form pink colonies on MacConkey agar. In the case of JP995, resistance to sulphonamide was tested on SAF containing 100 μg/ml of sulphadiazine.

Table 8. *Yearly consumption of certain anti-bacterial drugs in a general hospital in Hong Kong from* 1972 *to* 1976

	Yearly consumption (g)				
	1972	1973	1974	1975	1976
Ampicillin	134250	160250	202689	194750	225000
Streptomycin	9100	12600	7700	5700	5000
Tetracylcine	66377	65075	35050	35000	30000
Chloramphenicol	3900	2010	604	1830	2250
Kanamycin	328	200	100	230	0
Sulphadiazine	2420	2350	2592	600	500
Co-trimoxazale	3920	3680	2320	2480	4160
Gentamicin	710	714	938	1152	1263

respectively. In addition to this, transmissible factors carrying A.S.T.C.Su, A.T.C.K.Su and A or T resistance alone were also demonstrated from the donor *S. johannesburg* strains with the A.S.T.C.K.Su resistance pattern.

As only 1 out of the 138 *S. johannesburg* strains with the A.S.C.K.Su resistance pattern was sensitive to nalidixic acid at a concentration of 25 μg/ml (the other 137 strains were resistant to nalidixic acid even at concentrations higher than 50 μg/ml), a conjugation experiment could be performed only with this particular strain. Transmissible factors carrying resistance to A.S.C.K.Su *in toto* were detected from this donor strain when *E. coli* JP995 was used as the recipient strain. Again, transfer of resistance for streptomycin was not found, while transfer of resistance to other antibiotics was demonstrated when *E. coli* UB1139 was used as the recipient strain. The MICs of chloramphenicol of the donor Salmonella strain and the *E. coli* UB 1139 transconjugants were 320 and 80 μg/ml, respectively.

DISCUSSION

In this report, we have demonstrated the sequential change in chloramphenicol and ampicillin resistance in a single salmonella serotype, *Salmonella johannesburg*, endemic in a confined locality, Hong Kong. Two aspects of our observations on the multiple resistance of *S. johannesburg*, which has been prevalent in Hong Kong since 1973, merit special attention. The first is its high frequency of simultaneous resistance to chloramphenicol and ampicillin, the two important first-line drugs for the treatment of invasive salmonella infections. Furthermore, the resistance factors for ampicillin and chloramphenicol were transmissible and appeared to be linked with each other. Most of the *S. johannesburg* strains were isolated from faecal specimens and although the main trouble caused by them was diarrhoea, invasive infections did occur. These included osteomyelitis, local abscess, wound infection, septicaemia and urinary tract infection. Whether in the future *S. johannesburg* might pass its resistance factors to other Salmonella strains such as *S. typhi*, which is also endemic in this locality, is of practical importance for us to study. Fortunately, the majority of the *S. johannesburg* strains isolated remained sensitive to trimethoprim which, when used in combination with sulphamethoxazole, is an

effective drug in the treatment of invasive salmonellosis. Only 5 out of the 124 strains isolated in 1975, 1 out of the 118 strains in 1976 and none in 1977 were trimethoprim-resistant.

The second aspect of interest is that the change in the patterns of antibiotic resistance did not appear to correlate directly with the use of the relevant antibiotics. The consumption of both chloramphenicol and tetracycline in the general hospital concerned decreased over the 5-year-period of observation to about half the amount of that prescribed in the previous years (Table 8). Such a trend in the prescription of antibiotics held true also for the community at large, However, the proportion of *S. johannesburg* strains resistant to chloramphenicol steadily increased while that of strains resistant to tetracycline dramatically decreased, in spite of the fact that both chloramphenicol and tetracycline were rarely used for the treatment of salmonella gastroenteritis. A possible explanation of the increase in the proportion of chloramphenicol-resistant strains might be that resistance factors conferring chloramphenicol resistance code also for resistance to other drugs such as ampicillin. Selection of chloramphenicol-resistant strains thus could be due to the use of any other relevant antibiotic. If so, why did resistance to tetracycline disappear?

Four transmissible factors carrying tetracycline resistances have been demonstrated by studying some of the representative *S. johannesburg* strains. They were identified by co-transfer of resistance to other antibiotics: one carried A.S.T.C.K.Su, others carried A.S.T.C.Su, T.C and T alone. Over the 5-year period of observation, the tetracycline-sensitive strains showing the A.S.C.K.Su resistance pattern have superseded all the tetracycline resistant strains including those which carried a transmissible factor for T resistance alone. Are transmissible factors carrying tetracycline resistance probably a biological disadvantage to the host *S. johannesburg* cells in the absence of selective pressure by tetracycline? Or alternatively, does the transmissible factor carrying A.S.C.K.Su resistance offer to the host cells an additional advantage other than antibiotic resistance? It seems possible that factors other than antibiotics might play some role in the evolution of plasmids carrying such antibiotic resistance in *S. johannesburg* isolated in this locality.

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