Epidem. Inf. (1987) **98**, 379–383
Printed in Great Britain

Does chloramphenicol remain the drug of choice for typhoid?

BY B. ROWE, E. J. THRELFALL AND L. R. WARD

WHO Collaborating Centre for Phage typing and Resistance of Enterbacteria, Division of Enteric Pathogens, Central Public Health Laboratory, 61, Colindale Avenue, London NW9 5HT

(*Accepted 2 December 1986*)

SUMMARY

Of 2356 strains of *Salmonella typhi* isolated in Britain in the 8-year period 1978–85, 2345 (99·53 %) were sensitive to all antibiotics tested and 11 (0·47 %) were chloramphenicol-resistant; chloramphenicol resistance was plasmid-mediated in 6 strains. It is concluded that chloramphenicol remains a satisfactory first-line choice of drug for typhoid fever in Britain.

INTRODUCTION

It is necessary to start treatment for typhoid fever before the results of antimicrobial sensitivities are known and until 1972 chloramphenicol was the unquestioned drug of choice (Christie, 1980). However, the appearance of chloramphenicol-resistant *Salmonella typhi* in Mexico (Anonymous, 1972; Anderson & Smith, 1972), India (Paniker & Vimala, 1972) and Vietnam (Butler *et al.* 1973) gave rise to fears that strains resistant to chloramphenicol were becoming extensively distributed, thus jeopardising the supremacy of this antibiotic (Anonymous, 1973).

We report the incidence of chloramphenicol resistance in *S. typhi* isolated in Britain during the 8 years 1978–85. The implications of the results on the choice of drug for the treatment of typhoid fever in Britain are discussed.

MATERIALS AND METHODS

Phage typing and testing for antimicrobial drug resistance

A total of 2356 strains of *S. typhi* isolated in England, Wales and Scotland between January 1978 and December 1985 were phage-typed (Craigie & Felix, 1947) and screened for resistance to antimicrobial drugs. Strip-diffusion was used to test for resistance to ampicillin, chloramphenicol, gentimicin, kanamycin, streptomycin and tetracyclines, agar-dilution for resistance to sulphathiazole, trimethoprim, nalidixic acid and furazolidone (Anderson & Threlfall 1974).

Minimal inhibitory concentrations

When strains appeared resistant to ampicillin, chloramphenicol or tetracyclines, the minimal inhibitory concentrations (MICs) to these antibiotics were determined by observing the ability of approximately 10^3 cells/ml to grow at 37 °C for 18 h

in doubling dilutions of the respective antibiotics in nutrient broth, at concentrations ranging from 0·5 to 1000 μg/ml. When strains appeared resistant to trimethoprim, the MICs were determined by observing growth on nutrient agar plates containing concentrations of trimethoprim ranging from 0·5 to 1000 μg/ml together with 5% v/v lysed horse blood.

Transfer of resistance and characterization of plasmids

Strains resistant to chloramphenicol were tested for the ability to transfer resistance at 28 °C and 37 °C to a plasmid-free laboratory strain of *Escherickia coli* K12, nalidixic-acid resistant (= 14R525). Resistance plasmids were assigned to compatibility groups by standard methods (Anderson & Threlfall, 1974; Frost *et al.* 1983).

Isolation of plasmid DNA and agarose gel electrophoresis

All chloramphenicol-resistant strains were screened for plasmid DNA, using the methods of Birnboim & Doly (1979) and Kado & Liu (1981). Plasmid DNA isolated by these methods was visualized after electrophoresis on vertical slab gels containing 0·6% agarose. Molecular weights (MWs) were determined in relation to the mobility of four reference plasmids with MWs of 98, 42, 23·9 and 4·6 Megadaltons (MDa). These were carried in a single strain of *E. coli* K12, strain 39R861 (Threlfall *et al.* 1986).

RESULTS

Occurrence of chloramphenicol resistance

During the 8 years 1978–85, the majority of patients with *S. typhi* in England, Wales and Scotland had acquired their infections abroad, most commonly in the Indian subcontinent or West Africa. Of 2356 strains isolated from blood culture, 2345 (99·53%) were sensitive to all antibiotics tested. The isolates from 11 patients were chloramphenicol-resistant and comprised 0·47% of *S. typhi* examined (Table 1). These strains belonged to nine different phage types. Five of the patients with chloramphenicol-resistant *S. typhi* had been infected in India, two in Pakistan, two in the Middle East and one whilst travelling from Australia to Britain. The remaining patient gave no history of travel outside the British Isles.

In two further patients, chloramphenicol-resistant strains were isolated from faeces but only some time after the isolation of drug-sensitive strains from blood. The pathogenesis of typhoid fever is such that the *in vivo* acquisition of resistance in the gastrointestinal tract is of no clinical significance to the patients concerned. Drug-resistant strains isolated from faeces after the isolation of sensitive strains from blood will therefore not be discussed further here.

Resistance spectra (R-types)

Of the 11 chloramphenicol-resistant blood isolates, 6 were also resistant to ampicillin (A), 8 to tetracyclines (T) and 2 to trimethoprim (Tm). The complete antibiotic resistance spectra of these strains are shown in Table 1. With the exception of these 11 strains, no other antibiotic resistances were detected.

Table 1. *Chloramphenicol-resistant* Salmonella typhi *isolated from blood culture 1978–85*

Strains		MIC (μg/ml)			Plasmids	
R-type	No.	Chloram-phenicol	Trime-thoprim	Ampic-illin	Tetra-cycline	MW (MDa)	Inc group
CSSuT	3	125	< 0·5	4	125	120	H_I
ACT	1	125	< 0·5	125	125	92	$F_I me$
ACSSuT	1	250	< 0·5	125	125	98	$F_I me$
ACKSSuT	1	250	< 0·5	125	125	62	F_{II}
C	2	16	< 0·5	4	4	—	.
AC	1	8	< 0·5	8	4	—	.
ACTTm	1	32	8	16	16	—	.
ACSTTm	1	16	8	16	16	—	.
Total	11						

Resistance symbols: A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines; Tm, trimethoprim.

—, No plasmid species identified.

In parentheses, chloramphenicol-resistant *S. typhi* as percent of total *S. typhi* examined during the 8-year period.

Transfer of chloramphenicol resistance and characterization of plasmids

Resistance to chloramphenicol was transferable in six strains; in these strains the only plasmid DNA detected was that which corresponded in size to the DNA of the resistance plasmids after their transfer into 14R525. Three of the six strains with transferable resistance carried 120 MDa, Inc H_1 plasmids which coded for resistance to chloramphenicol, streptomycin, sulphonamides and tetracyclines (CSSuT) (Table); these strains were all isolated from patients infected in India. A strain from a further patient infected in India and a strain from a traveller infected in Pakistan each carried an Inc $F_I me$ plasmid; these plasmids were of 98 and 92 MDa and coded for resistance to ACSSuT and ACT respectively. Finally, the strain isolated from the person infected whilst travelling from Australia to Britain carried a 62 MDa, Inc F_{II} plasmid which coded for ACKSSuT (K, kanamycin).

In five strains, chloramphenicol resistance was non-transferable. No plasmid bands were detected when these strains were examined by agarose gel electrophoresis and the resistance was therefore presumed to be chromosomal. Two strains with non-transferable resistance were resistant to chloramphenicol only (C), one to ampicillin and chloramphenicol (AC), one to ampicillin, chloramphenicol, tetracyclines and trimethoprim (ACTTm) and one to ampicillin, chloramphenicol, streptomycin, tetracyclines and trimethoprim (ACSTTm) (Table).

Minimal inhibitory concentrations (MICs)

The MIC of chloramphenicol for strains with plasmid-mediated resistance ranged from 125 to 250 μg/ml and with strains with chromosomal resistance, from 8–16 μg/ml (Table). Similarly, the ampicillin MIC for the three strains with plasmid-mediated ampicillin resistance was 125 μg/ml whereas the MIC for the three strains with chromosomal ampicillin resistance ranged from 8–16 μg/ml. All

five strains with plasmid-mediated tetracycline resistance had MICs of 125 μg/ml to tetracycline. The two strains with non-transferable trimethoprim and tetracycline resistance had MICs of 8 μg/ml to trimethoprim and of 16 μg/ml to tetracycline.

DISCUSSION

These findings show that in Britain, chloramphenicol-resistant strains are unlikely to be isolated from persons with typhoid fever. This is suprising since the majority of typhoid cases in Britain have contracted their infections abroad and in particular in India and West Africa (Anonymous, 1985), areas in which chloramphenicol-resistant strains have either caused outbreaks or come to be regarded as endemic.

Chloramphenicol resistance was plasmid-mediated in 6 of the 11 chloramphenicol-resistant strains. Three of the strains with plasmid-mediated resistance, all isolated from patients infected in India, carried Inc H_1 plasmids. This is not unexpected, since *S. typhi* with H_1 plasmids have been reported as endemic in India (Sharma *et al.* 1979). However, two other plasmid compatibility groups were identified in the remaining three strains with plasmid-mediated resistance – F_I*me* (2 strains), and F_{II} (1 strain). Thus the claims that plasmid-mediated chloramphenicol resistance in *S. typhi* is almost invariably encoded by Inc H_1 plasmids (Farrar, 1985; Taylor *et al.* 1985) are incorrect, although H_1 plasmids have been reported on several occasions when chloramphenicol-resistant *S. typhi* have caused epidemics (Anderson, 1975). In the five strains with chromosomal resistance, the low levels of resistance are consistent with alterations in the permeability of the cell wall and not with the production of antibiotic-inactivating enzymes. The mechanism of antibiotic resistance in these strains is under investigation.

The high level of chloramphenicol resistance (125–250 μg/ml) in strains with plasmid-mediated resistance would certainly have rendered this antibiotic ineffective in clinical treatment; even the much lower levels of chromosomal resistance (8–32 μg/ml) might affect the therapeutic response. It has been recommended that co-trimoxazole should be used if chloramphenicol resistance is known or suspected (Garrod *et al.* 1981), but two of the chloramphenicol-resistant strains were also resistant to trimethoprim, with MICs of 8 μg/ml. Although these strains were sensitive to sulphonamides, it is possible that the trimethoprim resistance might affect the clinical response to co-trimoxazole. Unfortunately, these strains were also resistant to ampicillin with an MIC of 16 μg/ml and in these circumstances, treatment with cephalosporin antibiotics such as cefamandole (Uwaydah *et al.* 1984) or ceftriaxone (Ti *et al.* 1985) could be considered.

It is reassuring to know that chloramphenicol remains a satisfactory first-line drug of choice for typhoid fever in Britain and it should certainly be used whilst awaiting results of laboratory sensitivity tests.

REFERENCES

Anderson, E. S. (1975). The problem and implications of chloramphenicol resistance in the typhoid bacillus. *Journal of Hygiene* **74**, 289–299.

Anderson, E. S. & Smith, H. R. (1972). Chloramphenicol resistance in the typhoid bacillus. *British Medical Journal* iii, 329–331.

ANDERSON, E. S. & THRELFALL, E. J. (1974). The characterization of plasmids in the enterobacteria. *Journal of Hygiene* **72**, 471–487.

ANONYMOUS (1972). Typhoid fever – Mexico. *Morbidity and Mortality Weekly Report* **21**, 177–178.

ANONYMOUS (1973). Chloramphenicol resistance in typhoid. *Lancet* ii, 1008–1009.

ANONYMOUS (1985). Typhoid and paratyphoid fevers. OPCS Monitor 1985; MB2 85/2: 9–11. Office of Population Censuses and Surveys, 1985.

BIRNBOIM, H. C. & DOLY, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Research* **7**, 1513–1523.

BUTLER, T., LINH, N. N., ARNOLD, K. & POLLACK, M. (1973). Chloramphenicol-resistant typhoid fever in Vietnam associated with R factor. *Lancet* ii, 983–985.

CHRISTIE, A. B. (1980). Typhoid and paratyphoid fevers. In *Infections Diseases: Epidemiology and Clinical Practice*, 3rd edn. Edinburgh, London, Melbourne, New York: Churchill Livingstone.

CRAIGIE, J. & FELIX, A. (1947). Typing of typhoid bacilli with Vi bacteriophage. *Lancet* i, 823–827.

FARRAR, W. E. (1985). Antibiotic resistance in developing countries. *Journal of Infectious Diseases* **152**, 1103–1106.

FROST, J. A. THRELFALL, E. J. & WILLSHAW, G. A. (1983). Methods of studying transferable resistance to antibiotics *in vitro*. In *Antibiotics: Methods of Assessing Antimicrobial Activity and the Occurence of Resistance* (ed A. D. Russell & L. S. Quesnel) pp. 265–284. SAB Technical Series.

GARROD, L. P., LAMBERT, H. P. & O'GRADY, F. (1981). Infections of the alimentary tract. In *Antibiotics and Chemotherapy*, 5th edn. Edinburgh, London, Melbourne, New York: Churchill Livingstone.

KADO, C. I. & LIU, S.-T. (1981). Rapid procedure for detection of large and small plasmids. *Journal of Bacteriology* **145**, 1365–1373.

PANIKER, C. K. J. & VIMALA, K. N. (1972). Transferable chloramphenicol resistance in *Salmonella typhi*. *Nature, London* **239**, 109–110.

SHARMA, K. B., BHAT, M. B., PASRICHA, A. & VAZE, E. (1979). Multiple antibiotic resistance among salmonellae in India. *Journal of Antimicrobial Chemotherapy* **5**, 15–21.

TAYLOR, D. E., CHUMPITAZ, J. C. & GOLDSTEIN, F. (1985). Variability of Inc H_{11} plasmids from *Salmonella typhi* with special reference to Peruvian plasmids encoding resistance to trimethoprim and other antibiotics. *Antimicrobial Agents and Chemotherapy* **28**, 452–455.

THRELFALL, E. J., ROWE, B., FERGUSON, J. L. & WARD, L. R. (1986). Characterization of plasmids conferring resistance to gentamicin and apramycin in strains of *Salmonella typhimurium* isolated in Britain. *Journal of Hygiene* **97**, 419–426.

TI, T.-Y., MONTEIRO, E. H., LAM, S. & LEE, H.-S. (1985). Ceftriaxone therapy in bacteremic typhoid fever. *Antimicrobial Agents and Chemotherapy* **28**, 540–543.

UWAYDAH, M., NASSAR, N. T., HARAKEH, H., VARTIVARIAN, S., TALHOUK, A. & KANTARJIAN, H. (1984). Treatment of typhoid fever with cephamandole. *Antimicrobial Agents and Chemotherapy* **26**, 426–427.