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Drug resistance in relation to use of silver sulphadiazine cream in a burns unit

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SUMMARY Topical chemoprophylaxis of extensive burns with silver sulphadiazine cream led to a large increase in the proportion of sulphadiazine-resistant Gram-negative bacilli in a burns unit. When all sulphonamide treatment in the ward was stopped, the incidence of sulphonamide-resistant strains fell back to levels similar to those recorded when silver sulphadiazine treatment was introduced. This was associated with a large reduction in the incidence of resistance of certain Gram-negative bacilli (especially Klebsiella sp) to several antibiotics.

Transferable resistance to sulphadiazine, shown by conjugation experiments with *Escherichia coli* K12, was found in a majority of the strains of Klebsiella sp tested, and in some other species. A pattern of transferable resistance to tetracycline, cephaloridine, chloramphenicol, ampicillin, carbenicillin, and sulphadiazine (T Ce Cl A Ca S) was found in four of the 22 strains of Klebsiella tested, and closely related patterns were transferred by five other strains. These patterns of resistance were commonly found in Klebsiella sp isolated from burns in the period before the withdrawal of sulphonamides from the ward but were found in none of the Klebsiella strains isolated in the first six months after that period. Strains of Acinetobacter and Proteus, in which transferable resistance was not found, showed no appreciable fall or rise in sulphadiazine resistance; there was no fall in resistance of these organisms to tetracycline, cephaloridine, chloramphenicol, ampicillin or carbenicillin on withdrawal of sulphonamides from the ward, but there were substantial falls in resistance of Acinetobacter to kanamycin, gentamicin, trimethoprim, and tetracycline which were probably not caused by the withdrawal of sulphonamides.

Topical chemoprophylaxis is shown to have great value in the protection of severely burned patients against infection. An important requirement for routine prophylactic applications is stable sensitivity of the main pathogens to the agents applied. Most antibiotics fail in this respect, but polymyxin and certain antiseptics, notably silver nitrate and chlorhexidine, have been used in burns units without evidence of any significant emergence of resistance (Jackson *et al.*, 1951; Cason and Lowbury, 1960; Cason *et al.*, 1966). Silver sulphadiazine cream has been widely used, and though resistance to sulphonamides seemed likely to emerge if this agent was used for routine prophylaxis, this was not reported in various trials of silver sulphadiazine cream (Fox *et al.*, 1969; Stanford *et al.*, 1969; Hummel *et al.*, 1970), including our own trials on smaller burns (Lowbury *et al.*, 1971a and b). However, a recent trial of silver sulphadiazine cream in extensively burned patients, which showed it to have some prophylactic advantages over 0·5% silver nitrate compresses, also showed that a large proportion of the Enterobacteriaceae in burns became highly resistant to sulphadiazine during the trial; this was associated with a reduced prophylactic effectiveness of silver sulphadiazine cream (Lowbury *et al.*, 1976).

In this paper we describe the changes in sensitivity patterns of Enterobacteriaceae to sulphadiazine and other antimicrobial agents before and after withdrawal of silver sulphadiazine cream, the sulphonamides, and co-trimoxazole from use in the Burns Unit.

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Resistance of Enterobacteriaceae from burns to sulphonamides and other antimicrobial agents

MATERIAL AND METHODS

Gram-negative bacilli were isolated from burns as described elsewhere (Lowbury *et al.*, 1976). One strain of every colony type isolated per patient per

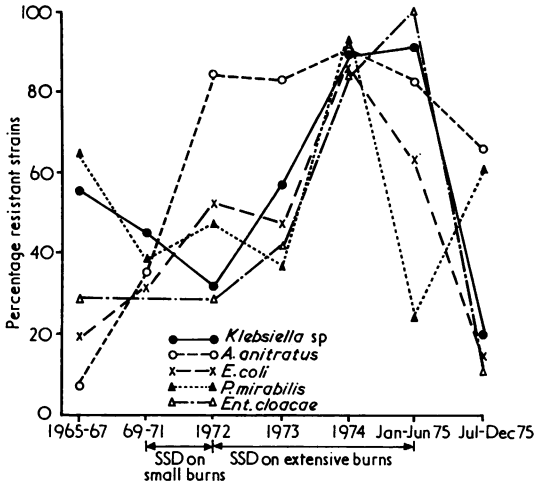

Figure *Percentage of strains of certain Gram-negative bacilli isolated from burns between 1965 and 1975 which were resistant to sulphadiazine. The periods in which silver sulphadiazine (SSD) was used and after which all sulphonamides were withdrawn are shown.*

month (or, in Jan-June 1974, per fortnight) was picked, identified by standard methods (Cowan and Steel, 1965), and tested for sensitivity to sulphadiazine, trimethoprim, and a range of antibiotics (see below) by a ditch plate technique (Topley *et al.*, 1951). The medium used for tests of sensitivity to sulphadiazine and trimethoprim was 4% Oxoid Diagnostic Sensitivity Test Agar containing lysed blood. Horse blood agar containing 4% New Zealand Agar was used for testing the nine other agents. Antibiotics and other antimicrobials were added to the agar medium, which was poured, after mixing, into ditches cut out from opposite sides of the agar plates. The following concentrations (μg per ml) of antibiotics were used: ampicillin, 125; carbenicillin, 100; sulphadiazine, 100; cephaloridine, chloramphenicol, gentamicin, kanamycin, nalidixic acid, tetracycline, and streptomycin, 50; trimethoprim, 10. These concentrations had been selected on the basis of tests previously made in parallel with tube or plate dilution tests; concentrations were chosen which allowed strains shown to be resistant by the dilution tests to grow up to or across the antibiotic ditch. A sensitive control strain of *Escherichia coli* was inoculated on each ditch plate. A selection of sulphadiazine-resistant strains was tested for minimal inhibitory concentration of sulphadiazine by a plate dilution test.

RESULTS

The Figure shows the proportion of strains isolated from burns of five species of Gram-negative bacilli that were resistant to sulphadiazine during a period covering three trials of silver sulphadiazine and a subsequent period of six months when silver sulphadiazine or sulphonamides were not used. These drugs were withdrawn because of the emergence of a very high incidence of sulphonamide-resistant Enterobacteria during the trial of silver sulphadiazine on extensive burns (Lowbury *et al.*, 1976).

The withdrawal of sulphonamides was associated with a prompt and, in the case of Klebsiella sp, *E. coli*, and *Enterobacter cloacae*, very large reduction in the proportions of sulphonamide-resistant strains; *Proteus mirabilis*, by contrast, showed an increase in sulphadiazine resistance at the same time. The reversion to previous levels of sulphonamide sensitivity may have been accelerated by the closure of the burn wards for approximately two weeks after the isolation of *Salmonella typhimurium* from the faeces of a patient with diarrhoea.

Tables 1, 2, 3 and 4 show the proportions of Klebsiella sp, miscellaneous Enterobacteriaceae (*E. coli*, Enterobacter sp, etc), *Acinetobacter anitratus*, and Proteus sp from burns which were resistant to each of the 11 antimicrobial drugs on which tests were done during the years 1974 and 1975. Treatment with silver sulphadiazine cream and sulphonamides was stopped in June 1975 because of the high incidence of sulphonamide resistance in the Burns Unit. There was a fall in the proportions of Klebsiella resistant to trimethoprim, kanamycin, streptomycin, and gentamicin in the first half of 1975 (before withdrawal of sulphonamides), and in the second half of 1975 there was a fall in the numbers of strains resistant to sulphadiazine, tetracycline, ampicillin, cephaloridine, chloramphenicol, and

Table 1 *Percentage of strains of Klebsiella spp resistant to antimicrobial drugs, 1974 and 1975*[1]

Antimicrobial drug	*Percent of resistant strains in:*			
	1974		*1975*	
	Jan-Jun	*Jul-Dec*	*Jan-Jun*	*Jul-Dec*
Sulphadiazine	94	88	92	20
Trimethoprim	35	26·5	4	0
Kanamycin	17	32	4	0
Streptomycin	35	26	8	0
Gentamicin	8·5	18	4	0
Tetracycline	90	94	96	20
Ampicillin	89	68	87·5	20
Cephaloridine	63	59	87·5	33
Carbenicillin	86	71	87·5	33
Chloramphenicol	84·5	53	87·5	0
Nalidixic acid	32	29	17	0
Total strains	71	34	24	15

[1]Sulphonamides were not used in the Burns Unit after June 1975

Table 2 *Percentage of strains of miscellaneous Enterobacteria resistant to antimicrobial drugs (1974-75)*

Antimicrobial drug	*Percentage of resistant strains in:*			
	1974		*1975*	
	Jan-Jun	*Jul-Dec*	*Jan-Jun*	*Jul-Dec*
Sulphadiazine	80	75	54	12
Trimethoprim	25	13	17	6
Kanamycin	8·5	16	11	4
Streptomycin	38	40	34	6
Gentamicin	2	4	3	1
Tetracycline	84	82	74	54
Ampicillin	65	55	69	27
Cephaloridine	70	62	54	59
Carbenicillin	16	26	31	10
Chloramphenicol	21	20	23	10·5
Nalidixic acid	10	8	3	7
Total strains	112	90	35	95

Table 3 *Percentage of strains of* Acinetobacter anitratus *resistant to antimicrobial drugs, 1974-75*

Antimicrobial drug	*Percentage of resistant strains in:*			
	1974		*1975*	
	Jan-Jun	*Jul-Dec*	*Jan-Jun*	*Jul-Dec*
Sulphadiazine	89	92	83	66
Trimethoprim	90	81	57	19·5
Kanamycin	61	52	66	5
Streptomycin	89	95·5	91	61
Gentamicin	56	49	57	4·9
Tetracycline	92	92	91	32
Ampicillin	87	90	63	71
Cephaloridine	97	98	97	80·5
Carbenicillin	6	10	11	10
Chloramphenicol	96	97	94	73
Nalidixic acid	42	44	40	56
Total strains	97	88	35	41

Table 4 *Percentage of strains of* Proteus spp. *resistant to antimicrobial drugs (1974-75)*

Antimicrobial drug	*Percentage of resistant strains in;*			
	1974		*1975*	
	Jan-Jun	*Jul-Dec*	*Jan-Jun*	*Jul-Dec*
Sulphadiazine	83	63·5	45	58·5
Trimethoprim	10	6	13	21·5
Kanamycin	9	2	0	0
Streptomycin	4	8	2	0
Gentamicin	0	0	2	0
Tetracycline	95	98	89	72
Ampicillin	10	8	15	8
Cephaloridine	15	19	13	29
Carbenicillin	5	4	0	6
Chloramphenicol	15	6	6	0
Nalidixic acid	6	8	4	1·5
Total strains	96	52	47	65

carbenicillin. Miscellaneous Enterobacteriaceae showed a progressive reduction in the proportions that were resistant to sulphadiazine, but there was less evidence of an associated fall in resistance to antibiotics. *A. anitratus* and Proteus sp showed little or no reduction (in the case of Proteus there was a slight increase) in sulphadiazine resistance after the withdrawal of sulphonamides, but *A. anitratus* showed a large reduction in kanamycin, gentamicin, trimethoprim, and tetracycline resistance in the second half of 1975.

A selection of resistant strains of several species tested by a plate dilution method was found to be highly resistant to sulphadiazine (minimum inhibitory concentration >1000 μg per ml).

Role of plasmids in the rise and fall of resistant Enterobacteriaceae in the Burns Unit

The fall in the proportion of strains (especially of Klebsiella) resistant to several antibiotics when sulphonamides were withheld suggested that sulphonamides were exerting a selection pressure that favoured strains with linked resistance to these agents. Such linked resistance is often transferable to sensitive Enterobacteriaceae in mixed culture. We examined strains of various species of Enterobacteriaceae isolated from burns during the period when sulphadiazine-resistant strains were predominant for transferable resistance patterns.

MATERIAL AND METHODS

A random selection of strains of *E. coli*, Klebsiella spp, Proteus spp, Enterobacter spp, Citrobacter spp, Serratia spp, and *A. anitratus* isolated in 1974 and 1975 was examined for the transfer of resistance patterns that included sulphadiazine.

Resistance transfer to *E. coli* K12 was tested with a strain of the recipient that was resistant to streptomycin or to nalidixic acid, depending on the sensitivity of the donor strains, growth of which was to be suppressed. One millilitre of 24-hour nutrient broth cultures of donor and recipient strains was added to 2 ml of nutrient broth, and the mixed culture was incubated for 24 hours at 37°C. It was then centrifuged at 3000 rev/min for 15 minutes, and the deposit was resuspended in 0·2 ml of nutrient broth, which was plated on nutrient agar containing 200 μg per ml sodium sulphadiazine and 200 μg per ml nalidixic acid or streptomycin; the plates were incubated at 37°C for 24 hours, after which colonies were picked, identified as *E. coli* by confirmatory biochemical tests, and tested for sensitivity to sulphadiazine, trimethoprim, kanamycin, gentamicin, streptomycin, ampicillin, cephaloridine, carbenicillin, tetracycline, chloramphenicol, and nalidixic acid by a ditch plate method (see above).

RESULTS

Table 5 shows the patterns of resistance transferred

Table 5 *Transferable resistance patterns including sulphadiazine in Gram-negative bacilli isolated from burns*

Bacteria from burns	*Number of strains tested*	*Number which transferred resistance*	*Resistance patterns transferred (and number of strains)*
E. coli	15	4	K A Ca S (1), A Ca S (1), T S (1), T Cl A Ca S (1)
Enterobacter sp	11	5	T S (1), St S (3), S (1)
Citrobacter sp	3	1	T K S (1)
Serratia sp	8	1	St Ce K A Ca S (1)
Klebsiella sp	22	14	K A Ca S (2), T Ce Cl A Ca S (4), Ce Cl A Ca S (2), T S (1), S (2), T Cl A Ca S (3)
Acinetobacter anitratus	20	0	
Proteus sp	12	0	—

T = tetracycline; K = kanamycin; Ca = carbenicillin; St = streptomycin; A = ampicillin; S = sulphadiazine; Ce = cephaloridine; Cl = chloramphenicol

Table 6[1] *Elimination of transferable resistance patterns on withdrawal of sulphonamide*

Year	*% Strains of Klebsiella sp showing resistance patterns T Ce Cl A Ca S, T Cl A Ca S, and Ce Cl A Ca S*	*Number of strains tested*
1972	14	44
1973	23	48
1974 Jan-Jun	66	71
1974 Jul-Dec	38	34
1975 Jan-Jun	79	24
1975 Jul-Dec	0	15

[1]See footnote to Table 5

to *E. coli* K12 by strains of Gram-negative bacilli from burns that were tested. Fourteen of the 22 strains of Klebsiella sp transferred resistance to the recipient strain, in contrast with no transfer of resistance from 20 strains of *A. anitratus* and from 12 strains of Proteus spp. The commonest pattern transferred by Klebsiella sp (4 strains) was tetracycline, cephaloridine, chloramphenicol, ampicillin, carbenicillin, and sulphadiazine (T Ce Cl A Ca S), but five strains transferred the closely related pattern Ce Cl A Ca S (2) and T Cl A Ca S (3).

The transfer of resistance plasmids from Enterobacter sp, Serratia sp, *E. coli*, and Citrobacter sp was also demonstrated.

Table 6 shows the proportion of isolates of Klebsiella sp from burns in the years 1972 to 1975 which showed the resistance patterns T Ce Cl A Ca S, T Cl A Ca S, and Ce Cl A Ca S. The frequency of isolation of strains showing these patterns rose from a low level in 1972 to a very high level in 1974 and in the first half of 1975, ie, during the period when sulphadiazine resistance had become predominant; in the six months after the withdrawal of sulphonamides and silver sulphadiazine from the Burns Unit, strains of Klebsiella sp showing these resistance patterns (the related patterns most commonly transferred by R factors) were no longer found in a series of strains tested. In the same period Klebsiella isolations became less common (for example, in the year 1974 there were 599 isolations of Klebsiella and in January to June 1975 there were 270 isolations, but only 59 isolations were recorded in July to December 1975). There had been an increase in isolations of Klebsiella strains resistant to chloramphenicol, tetracycline, cephaloridine, and ampicillin associated with the increase in sulphadiazine resistance during the period 1972-74 (see Table 7), but this increase was smaller than the fall which followed the withdrawal of sulphonamide treatment in June 1975.

Table 7 *Resistance of Klebsiella sp 1972-75*

Year	*Percentage of strains of Klebsiella resistant to*						*Total number o strains tested*
	T[1]	*Ce*	*Cl*	*A*	*Ca*	*S*	
1972	64	36	18	66	91	32	44
1973	48	56	35	77	69	56	48
1974 Jan-Jun	90	63	84	89	86	94	71
1974 Jul-Dec	94	59	53	68	71	88	34
1975 Jan-Jun	96	87	87	87	87	92	24
1975 Jul-Dec	20	33	0	20	33	20	15

[1]See footnote to Table 5

Discussion

The fall in sulphonamide, trimethoprim, and antibiotic resistance of Klebsiella after the withdrawal of sulphonamide treatment in the Burns Unit was associated with the disappearance from the Unit of Klebsiella strains with linked patterns of resistance to tetracycline, cephaloridine, chloramphenicol, ampicillin, carbenicillin, and sulphadiazine (T Ce Cl A Ca S), and to closely related patterns of resistance (T Cl A Ca S and Ce Cl A Ca S); these patterns were transferred to *E. coli* K12 in conjugation experiments. Their apparent elimination from the Unit was associated with a reduced incidence of Klebsiella infection of burns, a result reminiscent of that reported by Price and Sleigh (1970) in a neurosurgery ward where giving up the routine prophylactic use of streptomycin and ampicillin led to a great reduction

in the incidence of Klebsiella infections. Unlike the R factor RP_1, which was not removed from *Pseudomonas aeruginosa* and Enterobacteria in this Unit until all five antibiotics to which it determined resistance were withdrawn (Lowbury *et al.*, 1972; Roe and Lowbury, 1972), the R factor determining the resistance T Ce Cl A Ca S, T Cl A Ca S and Ce Cl A Ca S in Klebsiella spp was removed from the Unit by discontinuing the use of only one group of antimicrobials represented in the patterns (the sulphonamides).

The patterns of resistance and the response to the removal of selection pressure exerted by sulphonamide therapy were complex. Several different patterns of resistance were transferred by Klebsiella and Enterobacter sp. Proteus did not show the presence of R factors in the strains tested, and there was little or no change in the proportions of Proteus resistant to sulphadiazine, trimethoprim or the antibiotics on withdrawal of the sulphonamides from the Unit; but *A. anitratus*, which also showed no transfer of resistance to *E. coli* K12, appeared to respond to the withdrawal of sulphonamides in the ward by a large reduction in the proportions of strains resistant to trimethoprim, tetracycline, gentamicin, and kanamycin. But this association was probably coincidental and due to factors other than the withdrawal of sulphonamides, for there was little reduction in the proportion of sulphadiazine-resistant *A. anitratus* at the time when gentamicin, kanamycin, trimethoprim, and tetracycline resistance fell sharply. A large reduction in the proportions of Klebsiella resistant to trimethoprim, kanamycin, streptomycin, and gentamicin occurred in the six months immediately *before* the withdrawal of sulphonamides and was clearly due to factors other than sulphonamide usage.

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