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Multiple Transferable Drug Resistance in Enterobacteria in Mashonaland

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The development of resistance to antibiotics is so prevalent that it is prudent continuously to monitor the patterns of sensitivity in organisms likely to become involved. Particularly important are the staphylococci and the enterobacteria. The phenomenon is, with staphylococci, a problem found largely within hospitals and similar "close contact" institutions and, while the same may apply to some extent to the enteric organisms, increase in resistance in bacteria in non-hospitalised patients is becoming fairly common.

Particularly disquieting is the development of multiple resistance to antibiotics which frequently occurs through the medium of resistance transfer factors (R factors) from resistant to sensitive organisms. These factors, first recognised in 1959 by Ochiai *et al.*, and by Akiba in Japan in dysentery bacilli, are now known to occur in many species and to be transferable not only between similar species but also between certain genera. For example, transfer of this kind can take place between enterobacteria, pseudomonads, serratia, vibrios and pasteurella (Chabbert *et al.*, 1969). The potential clinical significance of the phenomenon is obviously very great.

In this study consecutively isolated strains of E. coli, some causing infection and others apparently commensals, and of shigella and salmonella were examined for the presence of drug resistance and to determine whether the resistance was due to chromosomal or extrachromosomal factors.

Material and Methods.

Bacteria

Coliform, salmonella and shigella organisms were isolated either from Rhodesian African in-patients at Harare Hospital, Salisbury, or from European patients from the Salisbury area. Initial isolations were made on MacConkey agar and identifications made by standard methods.

Resistance testing

Standard "mast" discs were used to test each strain for resistance or sensitivity to the following drugs: Chloramphenicol, ampicillin, tetracycline, kanamycin, cephaloridine*, streptomycin, gentamycin, neomycin and nalidixic acid.

Transferable resistance

Strains found to be resistant (donors) were mated with the recipient non-lactose fermenting E. coli 711 sensitive to all the above antibiotics except nalidixic acid. 0,5 ml each of overnight cultures of donor and recipient were inoculated into 4,5 ml of nutrient broth and incubated at 37° for 12-16 hours. As all donor strains were sensitive to nalidixic acid, 0,1 ml of the mixture was plated on to MacConkey medium containing this antibiotic and one other (seven plates in all). All antibiotics were incorporated at 100 ug/ml. Organisms growing on these plates were tested to confirm that they were recipient strains and were resistant.

Results.

E. coli.

Of the 78 consecutively isolated strains 37 were typable and 41 untypable.

Typable E. coli

Only five of the typable strains were fully sensitive to all the antibiotics tested (14 per cent.). Four were resistant to one antibiotic only (Strep. 2, Ceph. 1, Tetra 1) and one to two drugs only (Tetra. and Ceph.). The remaining 27 were resistant to three or more antibiotics. In eight of these the resistance was partly or wholly due to the presence of R. factors (22 per cent.) .

Table I shows the drug resistance of the typable E. coli isolates.

Table I.

Total	Chlor.	Amp.	Tetra.	Kana.	Ceph.	Strep.	Gent.	Neo.
37	25	27	25	20	11	21	0	5
%	68	73	68	54	30	57	0	13

Table II shows the resistance patterns in the typable strains having resistance to three or more antibiotics, and their sero types.

*Discs used late in the survey contained cephalothin rather than cephaloridine. Most organisms were tested against both drugs. Of 70 E. colis tested 34 were resistant to cephaloridine but only 15 to cephalothin.

Table II.

Resistance Pattern	*No. strains*	*Serotype*
Chlor, Ampi, Tetra, Kana, Strep, Neo.	3	111/B4
Chlor, Ampi, Tetra, Kana, Strep, Ceph.	3	111/B4
Chlor, Ampi, Tetra, Kana, Strep.	10	111/B4
Chlor, Ampi, Tetra, Kana, Neo.	1	114/K90
Chlor, Ampi, Kana, Strep, Neo.	1	111/B4
Chlor, Ampi, Tetra, Ceph.	5	111/B4, 125/B15
Chlor, Ampi, Tetra,	1	086/B7
Chlor, Ampi, Ceph.	1	125/B15
Ampi, Kana, Strep.	2	142/K86

Table III.

Resistance not transferred	*Resistance transferred*	*No. strains*	*Serotype*
Strep,	Chlor, Ampi, Tetra, Kana, Neo.	2	111/B4
Strep, Ceph.	Chlor, Ampi, Tetra, Kana.	3	111/B4
—	Chlor, Ampi, Tetra, Kana, Neo.	1	114/K90
—	Ampi, Kana. Strep.	1	142/K86

Table III shows the resistance patterns in the eight strains in which part or all of the resistance was due to R factors:

Non-typable E. coli

Five non-typable strains were fully sensitive to the antibiotics tested for (12 per cent.), one was resistant to two antibiotics and the remaining 35 were resistant to three or more drugs. Part or all of the resistance was due in 25 of the organisms to R factors (61 per cent.).

Table IV shows the drug resistance of the non-typable E. colis.

Table IV.

	Total	Chlor.	Ampi	Tetra.	Kana.	Ceph.	Strep.	Gent.	Neo.
	41	33	35	34	16	2	18	0	3
%		81	85	83	39	5	44	—	7

Table V shows resistance patterns in the non-typable strains.

Table V.

Resistance pattern	*No. strains*	*N.T.*
Chlor, Ampi, Tetra.	13	„
Chlor, Ampi, Tetra, Kana, Strep.	8	„
Chlor, Ampi, Tetra, Strep.	5	„
Chlor, Ampi, Tetra, Kana.	3	„
Chlor, Ampi, Tetra, Kana, Neo.	1	„
Chlor, Ampi, Tetra, Kana, Strep, Ceph.	1	„
Chlor, Ampi, Tetra, Kana, Strep, Ceph, Neo.	1	„
Chlor, Tetra, Kana, Strep.	1	„
Chlor, Tetra.	1	„
Ampi, Kana, Strep, Neo.	1	„
Ampi, Strep.	1	„

Table VI.

Resistance not transferred	*Resistance transferred*	*No. strains*
—	Chlor, Ampi, Tetra, Kana, Strep.	7
Strep, Ceph.	Chlor, Ampi, Tetra, Kana.	1
—	Chlor, Ampi, Tetra, Kana.	3
Strep.	Chlor, Ampi, Tetra, Kana.	1
—	Chlor, Ampi, Tetra.	10
Strep.	Chlor, Ampi, Tetra.	2
—	Chlor, Tetra.	1
Chlor, Tetra, Ampi, Kana, Neo.	Ampi,	1
Strep.	Ampi,	1

Table VII.

Resistance pattern	*No.*	*Type*
Chlor, Ampi, Tetra, Kana, Strep, Neo.	3	all dysenteriae
Chlor, Ampi, Tetra.	2	both flexner
Ampi, Tetra, Strep.	2	flexner, sonne
Ampi, Ceph.	1	boydii

Table VIII.

Resistance not transferred	*Resistance transferred*	*No. strains*
—	Ampi, Tetra.	1
—	Chlor, Ampi, Tetra.	2
Tetra	Chlor, Ampi, Kana.	2

Table VI shows resistance patterns in the 26 strains in which part or all of the resistance was due to R factors.

Shigella species

Over a six-month period 133 isolations of Shigella organisms were made from European and African patients both children and adults.

The strain were distributed as follows:

Strain	*No.*	*% of total*
Dysenteriae	13	10
Flexner	71	53
Boydii	13	10
Sonne	36	27

Of these 66 (50 per cent.) were fully sensitive to all the antibiotics tested for: 59 (44 per cent.) were resistant to one antibiotic only and only eight (6 per cent.) were resistant to two or more drugs.

In the single resistance group 56 were resistant to streptomycin and one each to ampicillin, tetracycline and cephaloridine.

The multiple resistance patters are given in Table VII.

Of the five strains available for testing for transferable resistance, four contained R factors.

Resistance transfer data is given in Table VIII.

Salmonella species

One hundred Salmonellas isolated randomly from European and African patients were examined. Forty-one were Salmonella typhi and 59 non-typhoid species.

All the typhoid organisms were fully sensitive to the antibiotics tested, as were 43 (73 per cent.) of the non-typhoid.

Two of the remainder were resistant only to tetracycline, and the 14 others had multiple resistance patterns as shown in Table IX.

Table IX.

Resistance pattern	*No. strains*
Ampi, Tetra, Strep, Ceph,	5
Ampi, Tetra, Strep.	4
Ampi, Strep.	3
Ampi, Strep, Ceph.	1
Neo, Kana.	1

In eight of these resistance was shown to be due in part or wholly to R factors. The results are summarised in Table X.

Table X.

Resistance not transferred	*Resistance transferred*	*No. strains*
Ampi, Strep.	Tetra.	2
—	Ampi, Strep.	2
Strep.	Ampi, Tetra.	2
Ampi, Strep.	Tetra.	2

DISCUSSION.

A very high proportion (88 per cent.) of all *E. coli* isolated whether from urine or faeces are resistant to one or more antibiotics, irrespective of whether they were recognisable as pathogenic serotypes or not. Of these 91 per cent. were resistant to three or more antibiotics. Combined resistance to chloramphenicol, ampicillin and tetracycline was found in over 70 per cent. of all strains with kanamycin and streptomycin resistance in approximately 45 per cent. No strains resistant to gentamycin were found and incidence against cephaloridine and neomycin was comparatively low.

Rather more of the non-typable strains carried resistance factors than did the typable (61 per cent. and 22 per cent., respectively). Patterns of transfer were, however, very similar between the two, the commonest combination being chloramphenicol, ampicillin, tetracycline and kanamycin. Resistance to streptomycin was rarely associated with R factors.

In contrast since screening began in 1968, very few multiply resistant Shigellas and Salmonellas have been found. No resistant typhoids have yet been isolated and only 14 of the 59 other species of Salmonella were resistant to more than one drug. The commonest resistant species were *Salm. barielly* and *Salm. newport,* and the commonest drugs ampicillin, streptomycin and tetracycline. The resistance was infectious in only eight (8 per cent.). Very few Shigellas were multiply resistant and of the strains tested, R factors were confined to four (3 per cent.).

These results with coliforms compare with those of Mare and Coetzee (1966) in Pretoria who found 78 per cent. of strains resistant and of Woods, Marcos and Hendry (1972) who found 82 per cent. resistance amongst the urban Xhosa though rather lower figures in Whites and in rural Xhosa. Hitherto *Salm. typhimurium* has been most frequently responsible for harbouring R factors and it has been but rarely isolated in Rhodesia. However, in surveys in Europe and the U.S.A. the incidence of R factors in Salmonella has varied from 18 to 95 per cent. Amongst Shigellas the incidence of multiple resistance has varied from place to place and year to year. Fluctuations from one to 12 per cent. are reported, for example, in Eastern Europe (Zajc-Satler, Grabner and Banic, 1969), and transferable resistance is found in from 60 to 90 per cent. of resistant organisms. Rhodesia must reckon itself fortunate that so few problems have yet arisen with Salmonellas and Shigellas.

Patterns of resistance both transferable and non-transferable are similar in our series as elsewhere (Mitsuhashi, 1971).

These findings pose certain real therapeutic problems and suggest other rather greater ones. The development of transferable resistance is undoubtedly favoured by indiscriminate administration of antibiotics both to man and to animals to whom they are fed to increase yield (Walton, 1966). The extent of the problem in animal enterobacteria has not yet been investigated in Rhodesia. It can, however, be seen that in the coliform multiple resistance is the rule and the planning of therapy, particularly without close bacteriological control, must bear resistance patterns in mind. That little if any transfer between genera takes place in vivo has surprised workers generally as in vitro it is readily demonstrable. Until the phenomenon is explained this additional possible hazard must be continually under laboratory surveillance. What has been clearly demonstrated, however, is that R factor carrying pathogens (and in virtually all the typable *E. coli* in this series were known pathogens), are more virulent in terms of morbidity and mortality than are those which do not. There is no doubt that where the dominance of enterobacteric carrying R factors is established, there are real dangers that can possibly be minimised by much stricter control generally in the handling of antibiotics.

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