INCIDENCE OF pGS01 AND pGSO4 TYPE SULPHONAMIDE RESISTANCE GENES AMONG CLINICAL ISOLATES COLLECTED FROM HOSPITALS IN SRI LANKA

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SYNOPSIS

Out of sixty three sulphonamide resistance clinical isolates, twenty four were found to carry pGS01 type, whereas, thirty three carried pGS04 type which do not cross hybridize. Five strains were found to carry both pGS01 and pGS04 type genes. All strains carrying pGS01 resistance carried a plasmid of size more than 50 kb except one, which carried plasmid of molecular size 35 kb. Among a multi-plasmid isolate (G 42) only plasmid pTVG42c (4.6 kb) carried pGS04 type gene. In another multi-plasmid isolated (G 39) the labelled probe of pGS04 gene was found to hybridize with both plasmids and the chromosomal DNA. Five isolates carried both types of sulphonamide resistance. One isolate carried sulphonamide resistance which did not hybridize with either of the above types.

INTRODUCTION

Plasmid mediated sulphonamide resistance are at least of two distinct types, each specifying a different type of dihydropteroate synthase (1). One of these type of plasmid borne sulphonamide resistance specifies a very labile dihydropteroate synthase activity that could be stabilised by the addition of sucrose. The same type of resistance is found in plasmid R1, R100, R6, R22259, R388, pGS01 and pGS02. This resistance is found to be associated with plasmid of size greater than 30 kb. The other type of plasmid borne sulphonamide resistance specifies a stable dihydropteroate synthase, the activity of which is not affected by the addition of sucrose. This resistance is found to be associated with plasmids pJM5SB, pJM25B, pGS03, pGS04, pGS05.

Sulphoramide is being widely used as the drug of choice for common ailments such as cough and fever in Sri Lanka. Hence, the incidence of different types of plasmid mediated sulphonamide resistance among various genomes carried by clinical isolates in this country could help to monitor their spread in the community at large.

MATERIALS AND METHODS

Sulphonamide resistance clinical isolates were collected from hospitals in Sri Lanka. One thousand and eight enteric aerobic gram negative bacteria isolated from clinical specimens (pus, urine, stools) at six bacterial diagnostic laboratories situated in different parts of the country were collected. They were collected over a period of 2 years and it is about 70% of the total strains isolated in different bacteriological laboratories in Sri Lanka from 1980 to 1982. Antibiotic sensitivity test and conjugation was carried out (2).

Screening for Plasmid DNA

Transconjugants carrying transferable antibiotic resistance were screened for the presence of plasmid DNA. (3) Plasmid DNA was precipitated by centrifuging at 15,6000 x g for 15 min in a Eppendorf centrifuge. The supernatant was drained off and the sediment was lyophilized at −40°C. The precipitate was resuspended in 30 µl of TE buffer (10 mM Tris hydrochloride: 50 mM EDTA).

Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out on a horizontal (0.5%) agarose gel slab at 40mA for 15 h. The apparatus was filled with Loening buffer (Tris acetate 40 mM, Sodium acetate 28 mM, Na_2 — EDTA 2 mM, pH 7.8). The gels were stained in freshly prepared ethidium bromide (2 µg/ml) for 30 min and was photographed under U.V. Light (wave length 254 nm) using a short wave transilluminator and polaroid type film.

DNA — DNA Hybridization

DNA-DNA hybridization was carried out separately using labelled probe of sulphonamide resistance genes in plasmid pGS01 an pGS04. These plasmid strains were supplied by Swedberg and Skold, Pharmaceutical Microbiology, University of Uppsala, Sweden. Fragments of sulphonamide resistance genes carried by plasmids pGS01 (1.3 kb *Hind*III — *Bam* H1 fragment) and PGS04 (1.0 kb EcoRI fragment) were labelled by nick translation separately (4).

Colony hybridization

Bacterial colony was picked by a sterile tooth pick and transferred to nitrocellulose paper. The cells were subjected to alkali lysis by NaOH and washed. Nitrocellulose paper was baked with the DNA and was hybridized with the labelled probe (5).

Southern blotting

Plasmid DNA was separated on a 0.5% agarose electrophoresis. The gel was trimmed and immersed in *soak* I solution (0.2 M NaOH, 0.6 M NaCl) for 45 mint. The gel was rinsed in water and immersed in *soak* II solution (1 M Tris pH 7.5, 0.6 M NaCl) for 45 min. The gels were sandwiched between filter paper maintained on an osmotic gradient in SSC solution overnight. The nitrocellulose filter papers were wrapped in tin foil and baked at 80°C for 2 h. The gels were checked for the absence of plasmid DNA bands. Nitrocellulose filter papers with the plasmid DNA were hybridized with labelled probes.

RESULTS

Total of sixty three sulphonamide resistant transconjugants wre screened for the presence of pGS01 and pGS04 type of sulphonamide resistance by colony hybridization. Twenty four transconjugants were found to carry pGS01 type of sulphonamide resistance whereas thirty three carried pGS04 type. Five transconjugants were found to carry both types of sulphonamide resistance genes (Tabze 1). One of the sulphonamide resistance transconjugants did not hybridize with any of these probes. Out of twenty eight sulphonamide resistant *Proteus* strains, seventeen were found to carry pGS04 type of resistance.

Sulphonamide resistance were found to be co-transferable with other resistance. pGS01 and pGS04 type were found to be co-transferable with ampicillin, tetracycline, chloramphenicol and trimethoprim resistance genes in thirteen and eight different combinations respectively (Table 2). Out of the twenty four isolates carrying pGS01 type of sulphonamide resistance nineteen

TABLE 1 INCIDENCE OF pGS01 and pGS04 TYPES OF SULPHONAMIDE RESISTANCE GENES AMONG ENTERIC SPECIES ISOLATED FROM CLINICAL SOURCE AND "HEALTHY" POPULATION

Bacterial species	No. of sulphonamide resistant transconjugants tested	No. of transconjugants carrying			
		pGS01 type of sulphonamide	pGS04 type of sulphonamide	pGS01 and pGS04 types of sulphonamide resistance	Sulphonamide resistance other than pGS01 and pGS04 types
Klebsiella	11	4	6	1	0
Proteus	28	10	17	1	0
E. coli	12	4	7	1	0
Citrobacter	4	1	2	1	0
Providentia	3	2	0	0	1
Serratia	1	1	0	0	0
Enterobacter	3	2	1	0	0
Pseudomonas	1	0	0	1	0
Total	63	24	33	5	1

TABLE 2 BACTERIAL STRAINS AND PLASMIDS

Transconjugant	Original Host	Plasmids	Mol. size	Markers	G Gene Type
J7 (Ec1005)	*Providentia*	pTVJ7	58.5	Su Cm Tp	—
J107a (Ec1005)	*Ps. flourescens*	pTVJ107a	67.0	Su Tc Tp	01, 04
G39 (Ec1005)	*E. coli*	pTVG39a) pTVG39b) pTVG39b)	58.5) 53.0) 53.0)	Ap Tc Su Tp	01, 04
C122 (Ec1005)	*Klebsiella*	—	—	Ap Tc Su Cm	01, 04
C223 (Ec1005)	*Proteus*	—	—	Ap Tc Su Tp	01, 04
C260 (Ec1005)	*C. freundi*	pTVC260	35.0	Tc Su	01, 04
J56 (Ec1005)	*Prot. rettgeri*	pTVJ56	58.5	Su Cm Tp	01
J79 (Ec1005)	*Citrobacter*	pTVJ79a) pTVJ79b)	58.5) 21.5)	Su Cm Tp	01
J82 (Ec1005)	*E. coli*	pTVJ82	66.0	Ap Tc Su Cm Tp	01
Ku17 (Ec1005)	*K. aerugens*	pTVKu17a) pTVKu17b) pTVKu17c)	56.6) 41.0) 18.0)	Ap Su Cm Tp	01
Ka183 (Ec1005)	*Prot. rettgeri*	pTVKa183	56.0	Su Cm Tp	01
G14 (Ec1005)	*Ent. agglomerans*	pTVG14a) pTVG14b) pTVG14c) pTVG49d)	59.5) 53.0) 49.0) 31.0)	Ap Su Cm	01
G20 (Ec1005)	*K. pneumoniae*	pTVG20	59.0	Ap Tc Su Cm Tp	01
G30 (Ec1005)	*E. coli*	pTVG30a) pTVG30b)	58.5) 41.0	Ap Tc Su Cm	01
G55 (Ec1005)	*Prot. morgani*	pTVG55	58.5	Su Cm Tp	01
G105 (Ec1005)	*Prov. stuarti*	pTV105	58.5	Tc Su Tp	01
G179b (Ec1005)	*Prot. mirabilis*	pTVG179b	58.5	Su Cm Tp	01
G187b (Ec1005)	*Prot. morgani*	pTVG187b	60.0	Ap Su Cm Tp	01
G195a (Ec1005)	*K. pneumoniae*	pTVG195a	58.5	Su bCm Tp	01
G222 (Ec1005)	*Prot. vulgaris*	pTVG222	67.0	Ap Su Cm Tp	01
G240 (Ec1005)	*E. coli*	pTVG240a) pTVG240b) pTVG240c)	67.0) 45.5) 18.0)	Ap Tc Cm Tp	01
G286a (Ec1005)	*Ent. cloacae*	pTVG286a	58.5	Ap	01
G286b (Ec1005)	*Serratia marcescens*	pTVG286b	56.0	Ap Tp	01
C70 (Ec1005)	*Prov. stuarti*	pTVC70	67.0	Ap Su Cm Tp	01
C193 (Ec1005)	*Prot. mirabilis*	pTVC193	59.5	Ap Su Cm Tp	01
C215 (EC1005	*Prot. mirabilis*	pTVC215	69.0	Ap Su	01
C233 (Ec1005)	*K. pneumoniae*	pTVC233	56.0	Ap Tc Su	01
C235 (Ec1005)	*Prot. mirabilis*	pTVC235	67.0	Ap Su Cm Tp	01
C286 (Ec1005)	*Prov. stuarti*	pTVC286	59.5	Ap Su	01
Am15 (Ec1005)	*E. coli*	pTVAm15	56.0	Ap Su	01
J135 (Ec1005)	*Prot. mirabilis*	pTVJ135	59.0	Su Cm	04
Ka2 (Ec1005)	*Prot. mirabilis*	pTVKa2a) pTVKa2b) pTVKa2c)	59.5) 11.3) 5.0)	Su Cm	04
Ka10 (Ec1005)	*Prot. mirabilils*	pTVKa10	55.0	Ap Su Cm	04
Ka49 (Ec1005)	*Prot. mirabilis*	pTVKa49a) pTVKa49b)	57.0) 40.0	Su Cm	04
A67 (Ec1005)	*Klebsiella*	—	—	Ap Tc Su Cm	04
A70 (Ec1005)	*Klebsiella*	pTVKa70a) pTVKa70b) pTVKa70c)	66.0) 39.0) 18.2)	Ap Tc Su Cm	04
A73 (Ec1005)	*Proteus*	—	—	Ap Su Cm	04
G42 (Ec1005)	*K. pneumoniae*	pTVG42a) pTVG42b) pTVG42c)	74.0) 5.0) 4.6)	Ap Tc Su Cm	04
G46 (Ec1005)	*Klebsiella*	—	—	Ap Su Cm	04
G52 (Ec1005)	*K. pneumoniae*	pTVG52	68.0	Ap Tc Su	04

Transconjugant	Original Host	Plasmids	Mol. size	Markers	Gene Type
G63 (Ec1005)	*E. coli*	pTVG63a) pTVG63b)	67.0) 57.0)	Ap Tc Su	04
G80 (Ec1005)	*K. pneumoniae*	pTVG80	66.0	Ap Tc Su Cm	04
G96 (Ec1005)	*Proteus*	—	—	Ap Tc Su	04
G113 (Ec1005)	*E. coli*	pTVG113	64.0	Ap Tc Su Cm	04
G174 (Ec1005)	*Klebsiella*	—	—	Ap Su Cm	04
G221 (Ec1005)	*Ent. cloacae*	pTVG221	67.0	Ap Tc Su	04
C14 (Ec1005)	*Prot. mirabilis*	pTVC14	66.0	Ap Tc Su Tp	04
C16 (Ec1005)	*Prot. mirabilis*	pTVC16	66.0	Ap Tc Su Cm Tp	04
C19 (Ec1005)	*Prot. mirabilis*	pTVC19	66.0	Ap Tc Su Cm Tp	04
C34 (Ec1005)	*Proteus*	—	—	Ap Su Cm	04
C46 (Ec1005)	*Prot. mirabilis*	pTVG46	53.5	Ap Tc Su Cm Tp	04
C53 (Ec1005)	*Prot. mirabilis*	pTVC53	64.0	Ap Su Cm	04
C83 (Ec1005)	*E. coli*	—	—	Ap Tc Su	04
C91 (Ec1005)	*Prot. mirabilis*	pTVC91	64.0	Ap Su Cm	04
C145 (Ec1005)	*C. freundi*	pTVC145	74.0	Ap Tc Su Cm	04
C169 (Ec1005)	*C. freundi*	pTVC169	72.0)	Ap Tc Su	04
C171 (Ec1005)	*E. coli*	pTVC171a) pTVC171b)	67.0) 57.0)	Ap Tc Su	04
C176 (Ec1005)	*Prot. mirabilis*	pTVC176a) pTVC176b)	45.5) 42.0)	Su Cm	04
C175 (Ec1005)	*Proteus*	—	—	Ap Su Cm	04
C212 (Ec1005)	*Prot. mirabilis*	pTVC212	66.0	Ap Su Cm Tc Tp	04
C295 (Ec1005)	*Proteus*	—	—	Ap Su Cm	04
Ny14 (Ec1005)	*E. coli*	—	—	Ap Su	04
Ny65 (Ec1005)	*E. coli*	—	—	Ap Tc Su	04

were associated with trimethoprim resistance whereas out of thirty three isolates harbouring pGS04 type of resistance only five were associated with trimethoprim resistance. The five transconjugants which carried both pGS01 and pGS04 type exhibited five different combinations out of which only one was found to be associated with trimethoprim resistance.

Twenty four transconjugants carrying pGS01 type sulphonamide resistance were screened for the presence of plasmid DNA (Table 2). All these transconjugants carried a plasmid of molecular size 35 kb. Out of thirty three transconjugants carrying pGS04 type, eleven carried a single plasmid of molecular size greater than 50 kb whereas seven carried more than one plasmid. Transconjugant (G 42 EC 1005) carrying pGS04 type of resistance was found to carry two plasmids of 74 and 5 kb (Table 2). It was observed that the labelled probe of pGS04 resistance gene hybridized with the 5 kb plasmid (Fig. 1). Another transconjugant (G39 EC1005) carrying both these sulphonamide resistance was found to carry three plasmids (Table 2). It was observed that the labelled pGS04 hybridized with the plasmid DNA as well as the chromosomal DNA, suggesting a transposon carrying such resistance.

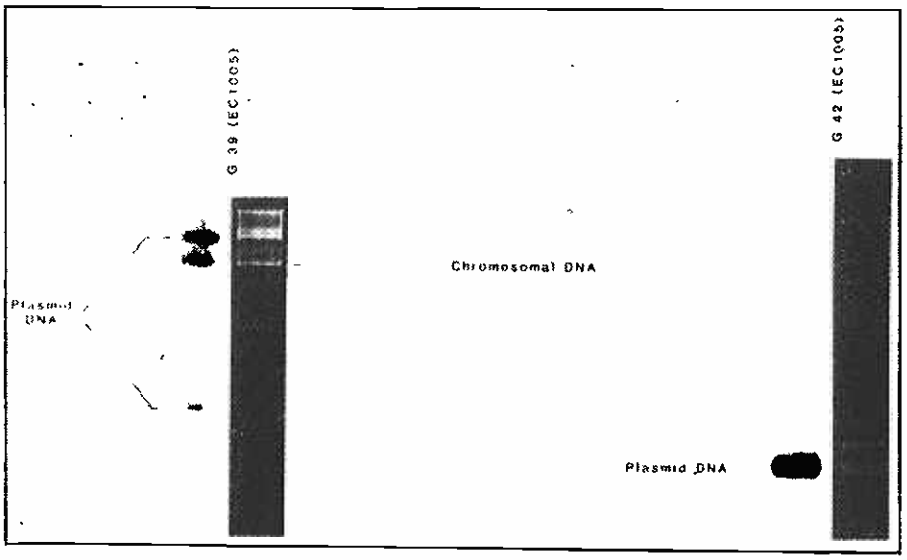

Fig. 1.
Autoradiograph of an agarose gel containing multiple plasmidsextracted from sulphonamide resistant transconjugant. Gel was probed with (^{32}p)-labelled DNA fragment containing pGS04 gene.

DISCUSSION

pGS01 type genes are usually harboured by plasmids of molecular size greater than 40 kb and pGS04 type is carried by plasmids of size less than 30 kb. However pGS04 type of gene could be found as a integral unit in large plasmids. (6) In spite of such diversity of the host genomes, the present study have shown that among clinical isolates pGS01 and pGS04 type of sulphonamide resistance genes are almost equally prevalent. This suggests that there is no preferential selection of these two genes by enteric bacteria. Five of the transconjugants were found to carry both pGS01 and pGS04 genes exhibiting heterogenous gene duplication. One of the transconjugants was found to carry neither pGS01 nor pGS04 type of sulphonamide resistance gene and probably it could be a third type of sulphonamide resistance gene. This has not been reported earlier and will be characterised by further studies.

Incidence of pGS04 type of resistance genes was found to be high among *Proteus*, showing tha the vector plasmid carrying pGS04 are likely to be mobilized more freely among this species. Integrated plasmids have a tendency to segregate into smaller units when harboured by *Proteus*. (7) Thus the pGS04 genes which is usually carried by smaller genomes are found among *Proteus*.

The preferential segregation of trimethoprim resistance genes with pGS01 is still obscure. Though pGS04 type of sulphonamide resistance has been found to be carried by both big and small plasmids it has never been reported to be carried on a transposon. Hence its presence on a transposon will enhance the spread of such resistance gene among the bacterial population.

Thus determination of different types of sulphonamide resistance genes among different genomes carried by differnt clinical isolate will help to monitor the spread of such genes in the community at large.

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