Phage Conversion in *Salmonella typhi*

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Abstract

Objective and background: This work aimed to convert Chloramphenicol resistant *S. typhi* into sensitive state genetically through transduction since Chloramphenicol resistant *S. typhi* is increasing all around the world.

Methodology: sixty patients were the subjects of this work. Each specimen collected from blood samples of having typhoid fever was subjected to well known microbiological methods for isolation and identification of *S. typhi* ,30 isolates of *S. typhi* were tested for sensitivity or resistance to the commonly used drug employing Kirby-Bauer technique, cross-lysis technique is followed to determine the sensitive isolates. Transduction was performed by mixing a phage lysates of certain isolates in separated system followed by expression on selective media. Antibiotics sensitivity test were performed looking for converted *S. typhi*.

Results: Out of 60 bloods collected from attending Sulaimayiah teaching hospital 30 isolates of *S. typhi* were obtained. Testing all isolates for antimicrobial susceptibility using Kirby-bauer disc diffusion method revealed that 93.3% of the isolates were Chloramphenicol resistant. Multidrug resistance in addition of Chloramphenicol have been observed in which the resistant pattern of *S. typhi* isolates showed Streptomycin is 86.6% ,Tetracycline 66.6%,Ampicillin 86.6%, Amoxicillin 80%, Ciprofloxacin 33.3% and Rifampicin 73.3%. Phages were induced [1] from Chloramphenicol sensitive isolates no 7 and 8. Employing cross-lysis [2] technique it has been found that isolates no.16 and 23 Chloramphenicol resistant *S. typhi* can be considered as indicator strains, In this work through application of transduction it was been found that Chloramphenicol resistant isolates no.16 and 23 were converted to Chloramphenicol sensitive ones following infection by phages induced from Chloramphenicol sensitive isolates no 7 and 8 respectively. Antibiotics sensitivity test were performed for all isolates treated with phage lysate looking for converted isolates.

Conclusion: It is concluded that Chloramphenicol resistant *S. typhi* were converted to Chloramphenicol sensitive *S. typhi* through transduction by phages from Chloramphenicol sensitive isolates.

Keyword: Transduction, Chloramphenicol resistance *S. typhi*

Introduction:-

S. typhi is widely distributed in our environment and responsible for a wide range of clinical conditions some of them are fatal if untreated properly due to mistake in diagnosis specially typhoid fever and meningitis. [3,4].

Many species are host-specific, those causing infection in man may not cause disease in animals and vice versa [4,5].

Enteric fever is mostly caused by strains of *S. typhi* and *S. paratyphi*, A, B or c in which clinical features tend to be more severe with *S. typhi* [5].

Characterization of strains has also been facilitated by determining their resistance to a range of antibiotics including Ampicillin, Chloramphenicol, Gentamicin, Kanamycin, Streptomycin, Sulfonamides, Tetracycline's and Trimethoprim [6,7].

Recently Chloramphenicol resistance by *S. typhi* is widely distributed over the entire world [8].

Bacteria are able to exchange genes in nature by three processes: conjugation, transduction and transformation and that bacteria usually develop their genes for drug resistance on plasmids so that they are able to spread drug resistance to other strains or species during genetic exchange process [9,10] Gene transfer is hampered in *S. typhi* and in other Pathogenic bacteria by the lack of a generalized transduction system. [11]. To transfer genes from *S. typhi* donors to *S. typhi* recipient an electroporation is substituted for generalized transduction as a method of genetic exchange [12,13,14].

Subjects & Methods:

Patients: Sixty patients were the subjects of this work from whom the blood samples were collected.

Methodology:

Specimen collection: Blood samples of 3-5ml were collected aseptically from each patient suspected of having typhoid fever.

Specimen processing: Blood culture technique in addition to culturing on MacConkey and S-S agar were applied.

Routine well established microbiological methods, for the diagnosis of S. typhi were applied. All 30 isolates were tested for their antibiotic susceptibilities and the resultant zones of growth inhibition were recorded according to an international value [1] as in table 1.

Phage induction: Ten ml of overnight cultures of Chloramphenicol sensitive *S. typhi* were held at water bath 56°C for 2h. Centrifuged at 3500 RPM for 15 minutes. Supernatants were collected to represent phage lysates. Cross lyses technique [2,24] is followed to determine the sensitive isolates of Chloramphenicol resistant isolates to certain phage lysate.

Phage conversion experiment: Transductions were performed by mixing a phage lysate of isolates of no 7 and 8 with an overnight culture of isolates no 16 and 23 in separate system followed by expression at 37°C for 1 hour prior to plating on selective

media[3]. Antibiotic sensitivity test were performed looking for converted *S.typi*.

Table 1: Interpretation of zone inhibition by using Kirby & Bauer method (disc diffusion method).

Antimicrobial	Code	Disc potency Mcg/ Disc	Diameter of zone inhibition		
			Resistant	intermediate	Sensitive
Ampicillin	AM	10	≤11	12-13	≥ 20
Cefotaxime	CTX	30	≤14	15-22	≥ 23
Cephalexine	KF		≤14	15-17	≥ 18
Chloramphenicol	C	30	≤12	13-17	≥ 18
Ciprofloxacine	CIP	10	≤15	16-20	≥ 21
Clindamycine	CN	2	≤12	13-17	≥ 18
Tobramycin	TM	10	≤13	13-14	≥ 15
Erythromaycin	E	15	≤13	14-17	≥ 18
Ampiclox	AMP	30	≤14	15-16	≥ 17
Gentamycin	GN	10	≤12	13-14	≥ 15
Nalidixic acid	NAL	30	≤13	14-18	≥ 19
Pencillin G	PG	6	≤20	21-28	≥ 20
Rifampicin	RA	5	≤16	17-19	≥ 20
Co-Trimoxazole	SXT	25	≤18	19-23	≥ 24-32
Amoxicillin	AMX	10	≤19		≥ 29
Amikcin	AN	30	≤14	15-16	≥ 17

Results:-

In this study 30 isolates of *S. typhi* out of 60 samples of blood were the subject of this study in which all isolates were subjected to antimicrobial sensitivity test of agar diffusion and transduction.

Susceptibility of an isolate of *S. typhi* to a certain antimicrobial is based on international values of the manufacture.

The following resistant pattern of *S. typhi* isolates was observed in which Chloramphenicol, 93.3%, Ampicillin, 86.6%, Amoxicillin, 80%, Ciprofloxacillin, 33.3% Streptomycin, 86.6%, Tetracycline, 66.6% and Rifampicin 73.3%.

Multidrug resistant *S. typhi* i.e resistance to 3 or more antibiotics was observed in this study.

Our attention have been focused on Chloramphenicol resistant *S. typhi* and attempt were done to convert Chloramphenicol resistant *S. typhi* in to sensitive one through genetic application of one of the main methods of gene transfer between bacteria i.e transduction. [11,12,13].

No conversion of Chloramphenicol resistant *S. typhi* into Chloramphenicol
sensitive one has been observed in the literature before our report.

In this study Chloramphenicol resistant *S. typhi* isolates no 16 and 23 were transduced into Chloramphenicol sensitive ones by infection by phages induced [2,3] from Chloramphenicol sensitive *S. typhi* isolates no. 7 and 8 respectively.

Discussion:-

It has been claimed by some workers [15,16,17] that resistance of *S.typhi* to Chloramphenicol 13%, Ampicilline, 13%, Amoxicillin, 0%, Ciprofloxacillin 10% and concluded that there is significant decrease over the years in resistance to Chloramphenicol was noticed.

Our results disagree with those results since it has been shown that there is increases in resistance of *S. typhi* to Chloramphenicol and other antibiotics, this is because as it is confirmed by others [16] who showed that resistance of *S. typhi* to Chloramphenicol is increasing Chloramphenicol resistant *S. typhi* were reported by several workers [17,18,19].

Since *S. typhi* is one of the most resistant organisms with multi-drug resistant strains. This could be to either due to abuse of drug or to widely distribution of plasmids which transfer drug resistant between bacteria.

Multi-drug resist *S. typhi* which is reported in this study seems to confirm other reports of other workers [20,21,22,23].

In this work Chloramphenicol resistant *S. typhi* were converted to sensitive ones by the action of phages from sensitive to Chloramphenicol *S.typhi* donors. The role of plasmids can be ruled out since plasmids transfer genes of resistance to antibiotic and not genes of sensitivity.

Gene transfer is hampered in *S. typhi* and other pathogenic bacteria by the lack of generalized transduction system.[11].

We over combed the problem of lack of generalized transducing system in *S. typhi* by the addition of few drops of 20% $Cacl_2$ solution to the phage bacterial culture mixture.[24,25].

Our work is supported by others [14,15] who showed that *S. typhi* v, phage were used to transduce temperature. Sensitive mutant of *S. typhi* and that antibiotic resistance and temperature-sensitive markers were transduced. [14]

It is concluded that Chloramphenicol resistant *S. typhi* were converted to Chloramphenicol sensitive *S. typhi* through transduction by phages from Chloramphenicol sensitive isolates.

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