## RESULTS

In the current study, 300 patients ranged in age from 2 to 60 years were suffering from signs and symptoms of typhoid fever. 170 of them were attended the out patients clinics of Mansoura Fever Hospital and 130 patients were attended Benha Fever Hospital. Out of 300 cases, 100 cases were diagnosed bacteriologically as typhoid fever and paratyphoid fever.

## 1- Results of bacteriologic examination

Table (1) shows the relation between the number of patients and the time of sample's collection in both hospitals. The number of cases with typhoid fever is more during August, followed by July, September and finally April. Typhoid fever was detected throughout the peroid of the study. It was more prevelant in August and July and report on resistance isolates.

<u>Table (2)</u> Shows the number of Salmonella isolated from Mansoura and Benha Fever Hospitals were 100 (33.3%) isolates. 60 isolates from Benha fever & 40 isolates from Mansoura Fever Hospital.

#### <u>Table (3)</u> Shows serotyping of isolated Salmonella

- \* 48 isolates were Salmonella typhi
- \*24 isolates were Salmonella paratyphi A
- \* 28 isolate swere Salmonella paratyphi B

<u>Table (1)</u> Salmonella infection among studied cases with suspected typhoid fever in different months.

Month	NO. of isolate	Salmonella isolate		Salmo resistant	
		NO.	%	NO.	%
	d				
April	19	10	10%	0	0 %
May	32	17	17%	1	4 %
June	43	15	15%	4	16 %
July	70	23	23%	8	32 %
August	79	25	25%	10	40 %
September	57	10	10%	2	8 %
Total	300	100	100%	25	100%

Salmonella incidence is high during August.

<u>Table (2)</u> Salmonella infection in Banha Fever Hospital and Mansoura Fever Hospital in the studied cases.

Hospital	Hospital Salmonella infecti	
	NO.	%
Benha Fever Hospital	60 from 130	46.1%
Mansoura Fever Hospital	40 from 170	23.5%

The total number of *Salmonella* isolated in both hospitals was 100 isolates. 60 isolates were isolated from Benha Fever Hospital and 40 isolates were isolated from Mansoura Fever Hospital.

<u>Table (3)</u> Serotyping of isolated Salmonella.

Serotyping	Number of Strains	%
Salmonella typhi	48	48%
Salmonella paratyphi A	24	24%
Salmonella paratyphi B	28	28%

# 2 - Antibiogram Results of The Isolated Salmonella

## <u>Table (4):</u> Showed the Antibiogram of isolated Salmonella and revealed that:

#### a) Salmonella typhi

- Strain number (24) was resistant to 6 antimicrobial chemotheraputic agents Streptomycin, Cephalothin, Cephradine, Chloramphenicol, Sulfamethiazole and Co-trimoxazole.
- Strain number 1, 2, 25 were resistant to 5 antimicrobial chemotheraputic agents. Strain number (1) was resistant to Cephradine, Cephalothin, Sulfamethiazole, Chloramphenicol and Cotrimoxazole while strain number (2) was resistant to cephalothin, cephradine, chloramphenicol, salfamethiazol and co-trimoxazol. (25) was resistant to Chloamphenicol, Cephaloxin, Oxytetracycline, Sulfamethiazole and Co-trimoxazole.
- Strain number (16), was resistant to four antimicrobial agents
   Oxytetracycline, Chloramphenicol, Sulfamethiazole, and Cotrimoxazole.
- Strain number 6,8,11, 13, 15, 19, 22 were resistant to three antimicrobial chemotheraputic agents Sulfamethiazole, Cotrimoxazole, and Chloramphenicol.

#### b) Salmonella paratyphi A

- Strain number (17) was resistant to 7 antimicrobial chemotheraputic agents viz., Streptomycin, Amikacin, Cephalothin, Cephrodine, Sulfamethazole, Co-trimoxazole and Chloramphenicol.
- Strain number (23) was resistant to 6 antimicrobial chemotheraputic agents Streptomycin, Cephalothin, Oxytetracycline, Chloramphenicol, Sulfamethazole and Co-trimoxazole.
- Four Strains were resistant to 3 antimicrobial chemotheraputic agents viz., Chloramphenicol, Sulfamethiazol and Co-trimoxazole isolates number 5, 18, 20, 21.

#### c) Salmonella paratyphi B

- Strain number (10) was resistant to 6 antimicrobial chemotheraputic agents which were Cephalaxin, Cefotoxim, Chloramphenicol, Sulfamethiazole, Co-trimoxazole and Naldexic acid.
- Strain number (12) was resistant to 5 antimicrobial chemotheraputic agents viz., Cephalaxin, Chloramphenicol, Sulfamethiazole, Cotrimoxazole and Naldexic acid.
- Two strains were resistant to 4 antimicrobial chemotheraputic agents. Strain number (4) was resistant to Chloramphenicol, Cephradine, Cephalaxin and Sulfamethiazole while Isolate number (7) was resistant to Chloramphenicol, Sulfamethiazole, Cotrimoxazole and Naldexic acid.
- Strains number (3), (9) and (14) were resistant to Chloramphenicol and Sulphamethiazole.

<u>Table(4)</u>:Antibiogram of Salmonella isolats

			1	Number	of Isolat	es		
Type of	1	2	3	4	5	6	7	8
Isolates⇒	S.typhi	S.typhi	Para	Para	Para	Typhi	Para	Typhi
	<b>.</b>		В	В	A		В	
ft								
S	+	++	++	+	++	+	++	+
PN	+	++	+++	+	+	+	++	++
PRL	+	+	+++	++	111	++	++	+++
KF	-	-	+++	+	++	+	+	++
CE	-	-	+++	-	++	++	+	+
CL	+	+	++		+++	+	+	++
CTX	++	+++	+++	++	++	+++	+++	++
AN	++	+++	+++	++	+++	++	++	++
GM	++	++	+++	++	+++	++	++	+++
К	1-1	+	++	++	+++	++	++	++
N	+	++	++	+	++	+	+	++
OFX	+++	+	++	+++	++	+++	+++	+
NOR	+++	++	+++	+++	+	+++	+++	++
OT	+	+	++	+	+++	++	+	++
С	-	-	-	-	-	-	-	-
TH	-	-	-	-		-	-	-
SXT	-	-	++	+	-	-	-	-
NA	+	+	+	+	+	+	-	++

Streptomycin PRL Piperacillin CE Cephradine CTX Cefotoxim PN Ampicillin KF Cephalothin

CL Cephalaxin

AN Amikacin **GM Gentamyicin** Kanamycin

**NOR Norfloxacin OT Oxytetracycline** C Chloramphenicol TH Sulfamethiazole

N Neomycin NA Naldixic Acid OFX Ofloxacin SXT Co-trimoxazole

(+) = Intermediate Sensitive (++)=Sensitive

(+++) =Highly Sensitive

<u>Table(4)</u> Antibiogram of Salmonella (follow)

	Number of Isolates							
Type of	9	10	11	12	13	14	15	· 16
Isolates⇒	Para	Para	Typhi	Para	Typhi	Para	Typhi	Typhi
	В	В		В		В		
↓						:		
S	++	++	+++	+	+	++	+	<del></del>
PN	+++	+	+++	++	++ .	++	++	+
PRL	++	+	++	+	+	++	+	+++
KF	+	+++	+	++	+++	+++	+++	+
CE	+	++	++	++	+	+++	++	++
CL	++	-	++	-	. ++	+++	++	++
CTX	++	-	++	++	++	+++	++	++
AN	++	++	++	++	++ .	+++	++	++
GM	+++	++	++	++	++	++-	++	++
K	+++	++	+++	++	++	++-	++	++
N	+++	++	+++	++	+++	++	++	+++
OFX	++	++	+++	++	++	+	+++	4++
NOR	++	++	+++	+++	+++	+	+++	+++
OT	++	++	++	++	++	++	++	•
С	=	-	-	-	-	_	-	-
TH	-	-	-	-	-	-	-	-
SXT	++	-	-	-	-	+	-	-
NA	++		+		. +	+	++	+

Table(4): Antibiogram of isolated Salmonella (follow)

	Number of Isolates								
Type of	17	18	19	20	21	22	23	3 24	1 25
Isolates⇒	Para	Para	Typhi	Para	Para	Typhi	Para	Typhi	Typhi
Antibiotic⊍	A	A		A	A		A		
S	-	+	+	++	+	++	-	-	+
PN	++	++	+	+++	+	++	++	++	+
PRL	+	++	++	+++	++	++	+++	+++	+
KF	-	++	· +	+++	+	+	-	-	+
CE	-	+++	+	+++	+	+	+	-	+
CL	++	+++	+	++	+++	++	++	++	-
CTX	+++	+++	++	++	+++	+++	+++	+++	+++
AN	-	+++	++	++	+++	+++	++	++	+++
GM	+++	++	++	+	+++	+++	++	+++	++
K	++	+	+	+	++	+++	+	++	1++
N	++	++	++	+	++	+++	+	+++	+++
OFX	+++	++	+++	++	+++	++	+++	+	4-4-
NOR	+++	++	+++	++	+++	++	++	+++	+++
OT	++	+++	+++	++	++	++	-	+	-
С	-	-	-	-	-	-	-	-	-
TH	-	-	-	-	-	-	-	-	•
SXT	-	-	-	-	-	-	-	-	-
NA	+	+	+	+	+	++	+	+	+

Table (4) shows that all Salmonella (25) isolates were susceptible to the following antibiotics: PRL (Piperacillin), GM (Gentamycin), K (Kanamycin), N (Neomycin) and OFX (Ofloxacin), NOR (Norfloxacin) and all isolates were resistant to Chloramphenicol and sulphamethiazol antibiotics.

<u>Table(5):</u> Number of Salmonella strains in relation to each antibiotics pattern.

Antibiotic	Resistant	Intermediate	Sensitive
		Sensitive	
Agents	NO%	NO%	NO %
S	3	11	11
PN	0	9	16
PRL	0	8	17
KF	5	10	10
CE	5	9	. 11
CL	4	5	16
CTX	1	0	24
AN	1	0	24
GM	0	1	24
K	0	5	20
N	0	6	19
OFX	0	3	22
NOR	0	2	23
OT	3	5	17
С	25	0	0
TH	25	0	0
SXT	21	2	2
NA	3	18	4

All *Simonella* strain were sensitive to GM, K, N, OFX and NOR and resistant to C, TH.

<u>Table(6):</u> The relation between the type of Salmonella and resistant marker

Type of	NO. of	Resistance Pattern	Number of resistant
Salmonellas	isolates		Marker
	1	CE,KF, TH, SXT, C	5
	2	KF, CE, C, TH, SXT	5
SALMONELLA	6	TH, SXT, C	3
TYPHI	8	C, TH, SXT	3
	11	C, TH, SXT	3
	13	C, TH, SXT	3
	15	C, TH, SXT	3
	16	OT, C, TH, SXT	4
	19	C, TH, SXT	3
	22	C, TH, SXT	3
	24	S, KF, CE, C, TH, SXT	6
	25	C, CL, OT, TH, SXT	5
	5	C, TH, SXT	3
SALMONELLA	17	C, KF, CE, TH, SXT,S,AN	7
PARA	18	C,TH, SXT	3
<i>ТҮРН</i> І А	20	C,TH, SXT	3
	21	C,TH, SXT	3
	23	S, KF, OT, C, TH, SXT	6
	3	C, TH	2
SALMONELLA	4	CE, CL, TH, C	4
PARA	7	C,TH, SXT, NA	4
<i>ТҮРНІ</i> В	9	C, TH	2
	10	CL, CTX, C, TH, SXT, NA	6
	12	CL, C, TH, SXT, NA	5
	14	C,TH	2

All 25 isolates were resistant from 2 to 7 antimicrobial agent.

## Results of Genetic Study

#### 1-Plasmid profile of Salmonella isolates

#### 1) Plasmid screening

All Salmonella isolates were screened for the presence of plasmids by agarose gel electrophoresis. The data of plasmid screening were summarised and shown in tables 7 and 8 and the photos of the gel electrophoresis No. (1, 2, 3).

#### a) - Result of Salmonella typhi

#### Table (7): Reveal the following

- \*Isolate number (6) contains 6 plasmids.
- \*Isolate number (13) contains 4 plasmids.
- \*Isolate number (16) contains 7 plasmids.
- \*Isolate number (24) contains 8 plasmids.
- \*Isolate number (19) contains 3 plasmids.
- \*Isolate number (22) contains 3 plasmids.
- \*Isolate number (25) contains 7 plasmids.
- \*Isolate number (15) contains 4 plasmids.
- \*Isolate number (8) contains 5 plasmids.
- \*Isolate number (11) contains 4 plasmids.
- \*Isolate number (1) contains 7 plasmids.
- \*Isolate number (2) contains 5 plasmids.
- \*All isolates of *Salmonella typhi* showed from three plasmids to 8 plasmids whight ranged between 7.1 to 1.2 M.D.
- \*Two isolates shared a common plasmid of molecular weight 5.2, 2.7,
- 1.7 M.D which are Isolates number 19 & 22.

\* Plasmids of molecular weight 2.7, 1.7 is present in all of the strain. It may responsible for resistance to C, TH.

#### b) - Result of Salmonella Paratyphi B

- \*Isolate number (10) contains 8 plasmids.
- \*Isolate number (12) contains 4 plasmids.
- \*Isolate number (4) contains 4 plasmids.
- \*Isolate number (7) contains 1 plasmids.
- \*Isolate number (3, 9, 14) were plasmid less.
- \* Four isolates showed one plasmid up to 8 plasmid their molecular weight ranged from 5.2 to 1.7. Isolates number (3,9,14) were plasmid less.

#### c) - Results of Salmonella Paratyphi A

- \*Isolate number (23) contains 4 plasmids.
- \*Isolate number (5) contains 2 plasmids.
- \*Isolate number (18) contains 1 plasmid.
- \*Isolate number (17) contains 2 plasmids.
- \*Isolate number (20) contain 1 plasmids.
- \*Isolate number (21) was plamid less plasmids.
- \* Five isolates showed one plasmid up to four plasmids their molecular weight ranged between 5.4 to 1.2.
- \* Two isplates shared a common plasmid of molecular weight 3.7 M.D. (Isolates number 23 & 5).

<u>Table (7)</u> The number & the size of plasmids extracted from 25 Salmonella isolates.

Type of strain	No.of strain	No.of plasmids	Plasmid Mol. wt. in (MD)
	. 6	6	7.1, 5.2, 3.7, 2.7, 1.7, 1.2
Salmonella	13	4	5.7, 3.7, 2.7, 1.7
Typhi	16	7	5.7, 3.7,3,2.7,1.7,1.5, 1.2
	24	8	5.7, 3.9, 3.7, 3, 2.7, 1.7, 1.5, 1.2
	19	3	5.2 , 2.7 , 1.7
	22	3	5.2 , 2.7 , 1.7
	25	7	5.7 ,3.7 ,3.5 ,2.7 ,1.8 ,1.7 , 1.5
	15	4	4.8, 2.7, 1.7, 1.2
	8	5	5.7, 4.8, 2.7, 1.7, 1.2
	11	4	3.7, 3.4 , 2.7 , 1.7
]	1	7	7.2, 5.2, 3.9, 3.5, 3, 2.7, 1.7
	2	5	5.2, 3.9, 3, 2.7, 1.7
	10	7	5.2, 4.4, 3.5, 3, 2.7, 2.2, 1.7
Salmonella	12	4	5, 4.3, 2.9, 2.6
Para	4	4	5.2, 4.2, 3.1, 1.7
<i>Typhi</i> B	7	1	1.7
	3,9,14	-	plasmid less
	23	4	3.7, 3.5, 3.1, 2.7
Salmonella	5	2	3.7 , 2.9
Para	18	1	3.9
Typhi A	17	2	2.7 , 1.2
	20	1	5.4
	21	_	plasmid less

From the previous table the approximate size of the plasmid in *Salmonella typhi* ranged from 7.1 to 7.1 and *Salmonella paratyphi B* ranged from 5.2 to 1.7 and 3 isolates were plasmid less and *Salmonella paratyphi A* ranged from 5.4 to 1.2 and one isolate was plasmid less.

## V5176 13 16 24 19 22 25 15 8 11 1 2

romosomal) chrom
5.2
3.5
3
1.7
1.5
1.2

Fig (1): Plasmid profile of resistant strains of Salmonella typhi lane

V517, molecular size standard (5.2, 3.5, 3, 1.7, 1.5, 1.2)

Lans 6, 13, 16, 24, 19, 22, 25, 15, 8, 11, 1,2, are resistant strains of Salmonella typhi. The number of bands from 2 to 8 bands each band indicate a plasmid.

chrom 5.2 3.5 3 1.7 1.5

Fig (2): Plasmid profile of resistant strains of Salmonella paratyphi B lane V517, molecular size standard Lans 7, 4, 3, 9, 10, 14, 12, are resistant strains of Salmonella paratyphi B the number of bands from 1 to 8 bands each one indicate a plasmid.

## V<sub>517</sub> 5 17 23 18 20 21

(chromosomal) chrom
5.2
3.5
3
1.7
1.5
1.2

Fig (3): Plasmid profile of resistant strains of Salmonella paratyphi A
lane V517, molecular size standard and lane 5, 17, 23, 18, 20,
21 resistant strains the number of bands from 2 to 4 and strain
number 21 is plasmid less

<u>Table (8):</u>Relation between plasmid molecular weight and number of plasmid

Plasmid Molecular Weight in M.d.	Number of plasmid	Frequency %
Salmonella typhi	:	
1.7	12	100 %
2.7	12	100 %
5.7	5	40.7 %
5.2	5	40.7 %
3.7	. 6	50 %
1.5	3	25 %
1.2	4	33.3 %
Salmonella Para B		
1.7	3	42.8 %
2.7	1	14.3 %
<u>Salmonella Para A</u>		
3.7	2	33.3 %
2.7	2	33.3 %

- \* A plasmid of molecular weight 1.7 & 2.7 M.d. are present in all strains of *Salmonella typhi* and in 2&3 strains of *Salmonella paratyphi B&A*.
- \* Plasmid of molecular weight 5.7 & 502 M.D. are present in 5 strains of Salmonella typhi.
- \* Plasmids of molecular weight 3.7 M.d. is present in 6 strains of Salmonella typhi and 2 strains of Salmonella paratyphi A.
- \* Plasmid of molecular weight 1.5 M.d. is present in 3 strains of Salmonella typhi.
- \* Plasmid of molecular weight 1.2 is present in 4 strains of Salmonella typhi.

## 2.Plasmid profiles and resistance patterns

#### in Salmonella isolates

- 1- The photos of agarose gel electrophoresis numbers 1, 2, 3, of 25 isolates of *Salmonella* with different resistance patterns, revealed that 21 of the isolates showed different plasmid profiles and 4 were plasmidless.
- 2- The plasmid profile and the resistance patterns of the individual isolate are shown in the table (10) in which the plasmid profile was very heterogeneous. There was no single profile that was common in two of the isolates.
- 3- With the increase of the resistance pattern to the antibiotic there was more increase in the number of isolates plasmids. For example, isolate number (24) which was resistant to 6 antibiotics harboured 8 plasmids and Isolate number (25) which was resistant to 5 antibiotics contained 7 plasmids, while strains 3,9,14,21 had no plasmids.

<u>Table(9):</u> Plasmid profile and resistance pattern of Salmonella isolates.

Type of strain	NO. of	Resistance pattern	Plasmid profile Mol ecular
	isolates		Weight(M.D)
	6	C,TH,SXT	7.1, 5.2, 3.7, 2.7, 1.7,1.2
	13	C,TH,SXT	5.7, 3.7, 2.7,1.7
SALMONELLA	16	OT,C,TH,SXT	5.7, 3.7,3, 2.7, 1.7, 1.5, 1.2
TYPHI	24	S,KF,CE,C,TH,SXT	5.7,3.9,3.7,3,2.7,1.7,1.5,1.2
:	19	C,TH,SXT	5.2, 2.7, 1.7
	22	C,TH,SXT	5.2, 2.7, 1.7
	25	C,CL,OT,T,SXT	5.7,3.7,3.5,2.7,1.8,1.7,1.5
	15	C,TH,SXT	4.8,2.7,1.7,1.2
	8	C,TH,SXT	5.7,4.8,2.7,1.7,1.2
	11	C,TH,SXT	3.7,3.4,2.7,1.7
	1	CE,KF,TH,SXT,C	7.2,5.2,3.9,3.5,3,2.7,1.7
	2	KF,CE,C,TH,SXT	5.2,3.9,3,2.7,1.7
SALMONELLA	10	CL,CTX,C,TH,SXT,NA	5.2,4.4,3.5,3,2.7,2.2,1.7
PARA	12	CL,C,TH,SXT,NA	5, 4.3, 2.9, 2.6
<i>TYPHI</i> A	4	CE,CL,TH,C	5.2,4.2,3.1,1.7
	7	C,TH,SXT,NA	1.7
	3,9,14	C, TH	plasmid less
SALMONELLA	23	C,TH,SXT,S,KF,OT	3.7,3.5,3.1,2.7
PARA	5	C,TH,SXT	3.7,3.1
<i>ТҮРНІ</i> В	18	C,TH,SXT	3.9
	17	C,KF,CE,TH,SXT	2.7, 1.2
	20	C,TH,SXT	5.4
	21	C,TH,SXT	plasmid less
	<u> </u>	······································	<del></del>

Plasmids of molecular weight 2.7, 1.7 are present in all strains of Salmonella typhi and in most strains of paratyphi A & B.

## 3. Transfer of plasmids

Salmonella isolates (21) harbouring plasmids were used by experimental trials to transfer their content to  $E.coli\ K_{12}$  which is sensitive strain to all antibiotics and resistant to naledixic acid. This was done by conjugation and transformation techniques.

#### Conjugation

The data of table (10) indicate that most of the detected plasmids were transferable by conjugation to the recipient strain. This is confirmed by the patterens of resistance of the donor and recipient strain which turned out to be identical. The rate of conjugation was comparable among the tested strains with a range from  $.3 \times 10^{-8}$  to  $1.3 \times 10^{-7}$ .

All resistant plasmids of *Salmonella* were transferred by conjugation to the sensitive strain E.coli K<sub>12</sub> which is naledixic resistant and the transconjugants colony apeared and can be counted on plates which contain sulfamethiazol(25ug) and co-trimoxazol(25ug) and nalidixic acid(50ug) calculated according to (MIC). The donor is resistant to sulfamethiazol and co-trimoxazol. The recipient is resistant to nalidixic acid and sensitive to all antibiotics. Transconjugant is strains resistant to sulfamethiazole and co-trimoxazol and nalidixic acid.

<u>Table (10)</u> Conjugation frequency of plasmid of Salmonella donor and corresponding E. coli K<sub>12</sub> transconjugants.

Donor	Number of	Conjugation
Designition	transconjugants	Frequency
1	4	4 x 10 <sup>-8</sup>
2	6	6 x 10 <sup>-8</sup>
6	7	7 x 10 <sup>-8</sup>
8	5	5 x 10 <sup>-8</sup>
11	9	9 x 10 <sup>-8</sup>
13	6	6 x 10 <sup>-8</sup>
15	8	8 x 10 <sup>-8</sup>
16	11	1.1 x 10 <sup>-7</sup>
19	3	3 x 10 <sup>-8</sup>
22	13	1.3 x 10 <sup>-8</sup>
24	4	4 x 10 <sup>-8</sup>
25	7	7 x 10 <sup>-7</sup>
20	5	5 x 10 <sup>-8</sup>

The data of table (10) and photos indicate that one or two plasmids were transferable by conjugation to the recipient E. coli  $K_{12}$ . The frequency of conjugation with the range of  $3x10^{-8}$  to  $1.3x10^{-7}$ .

#### **Transformation**

The data of the table (11) indicate that the number of the transformed cell is from 44 to 170 cells / plate. Transformation frequency ranged between  $1.1 \times 10^{-5}$  and  $2.5 \times 10^{-6}$  with an average of  $1.3 \times 10^{-6}$  and the resistance marker is positive by using antibiotic plates and by photo of transformed plasmid in agarose gel .

Since transformation is a known result in the transfer of one plasmid or more it is used in the present work for 21 isolates of Salmonella typhi and paratyphi A&B and a potent recipient strain E.coli K<sub>12</sub>.

The marker of the individual transformants was subjected to antibiotic susceptibility test using antibiotic disc and the result mentioned that all transformant cell produced by the individual donor gave indentical antibiotic susceptibility to the donor.

Fig (4): Agarose gel electrophoresis of plasmid DNA .Lane 1 plasmid less recipient E.coli K12 .Lane 2 donor Salmonella paratyphi A strain NO. 20 .Lane 3 transconjugant contain the same plasmid of donor cell .

Fig (5): Agarose gel electrophoresis of plasmid DNA .Lane 1 E.coli K12 .Lane 2 transconjugant .Lane 3 transconjugant . Lane 4 donor Salmonella typhi strain 8 .

 $1\overline{2}$  3

Fig (6): Agarose gel electrophoresis of plasmid DNA .Lane 1 transconjugant .Lane 2 donor Salmonella typhi 13.Lane 3 recipient E.coli K12

<u>Table (11):</u> Transformation rate and frequancy of the Salmonella isolates.

Isolate	Number of	Transformation
Designetion	Transformed Cell	Frequancy
Salmonella typhi		
1	120	6 x10 <sup>-6</sup>
2	160	8 x10 <sup>-6</sup>
6	144	7.2x10 <sup>-6</sup>
8	130	6.5x10 <sup>-6</sup>
11	170	8.5x10 <sup>-6</sup>
15	100	5 x10 <sup>-6</sup>
16	110	5.5x10 <sup>-6</sup>
19	74	3.7x10 <sup>-6</sup>
22	50	2.5x10 <sup>-6</sup>
24	66	3.3x10 <sup>-6</sup>
25	50	2.5x10 <sup>-6</sup>

The number of transformed *E.coli* C<sub>600</sub> cells counted on the plate was ranged from 50 to 170 and the transformation frequencies ranged from 2.5x10<sup>-6</sup> to 8.5x10<sup>-6</sup>

Table (11): follow

Isolate	Number of	Transformation
Designetion	Transformed Cell	Frequancy
Salmonella		·
paratyphi A&B		1
4	124	6.2 x10 <sup>-6</sup>
7	44	2.2 x10 <sup>-6</sup>
10	74	3.7 x10 <sup>-6</sup>
12	100	5 x10 <sup>-6</sup>
5	52	2.6 x10 <sup>-6</sup>
17	66	3.3 x10 <sup>-6</sup>
18	144	7.2 x10 <sup>-6</sup>
20	50	2.5 x10 <sup>-6</sup>
23	120	6 x10 <sup>-6</sup>

The transformation frequency of Salmonella paratyphi A&Salmonella Paratyphi B strains ranged from 2.2 x10<sup>-6</sup> to 7.2 x 10<sup>-6</sup>

1 2 3 4

Fig (7): Agarose gel electrophoresis of plasmid DNA of donor and transformed plasmid. Lane 1 donor strain 24 Salmonella typhi. Lane 2 recipient transformed cell. Lane 3 recipient transformed cell. Lane 4 donor strain 25 Salmonella typhi.

### DISCUSSION

In the present study, the monitoring of Salmonella genus as a causative agent of enteric, typhoid and paratyphoid fever has been conducted on patients attending the Out-Patient clinics of Benha and Mansoura Fever Hospitals. This study revealed that the incidence of resistant-Salmonella infection was 25 out of 100 cases (25%) of

typhoid fever.

The spread of multi-resistance strains, especially in countries of high endemi like Egypt, is a very serious consequences (*El-Bahrawi and Amin, 1989*).

By monitoring the results of susceptibility of *Salmonella* to the antimicrobial chemothrapeutic agents in this study, it has been noticed that 100 isolates, tested against (18) antimicrobial agents (25) isolates of them showing different type of resistance (25%), while 75 of the isolates were completely sensitive.

In the present study multiple drug registence to 2 to 7 or more