ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 04 | April - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

"A STUDY ON EMERGENCE OF MULTIDRUG RESISTANT SALMONELLOSIS"

Microbiology

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ABSTRACT

Background : Purpose: to screen Salmonella typhi in asymptomatic typhoid carriers and to find out drug resistance and ability of the strains to transmit drug resistance to other bacteria. Methods: Cultural characters, biochemical tests, antibiotic sensitivity test (disc diffusion), agarose gel electrophoresis, and conjugation protocols were done.

Conclusion: This study shows that drug resistant strains are able to transfer genes encoding drug resistance.

KEYWORDS

Asymptomatic typhoid carriers, antibiotics, drug resistance, plasmids, conjugation

INTRODUCTION

The ability to control infections with antibiotics has revolutionized the practice of medicine. Indeed, it has been suggested that the introduction of antibiotics has added ten years to an individual's life expectancy, (Donowitz and Mandell. 1988). Bacteria however, have responded to the development and clinical use of antibiotics with a bewildering array of resistance mechanisms. (Amyes and Gemmel,1992). Such adaptations have complicated the treatment of infections because resistant bacteria are associated with increased risk of morbidity and mortality (Holmberg et al 1987; Murray 1991).

Indiscriminate use of antibiotics is common in those countries where these are freely available over the medicine counter without prescription. Therefore, resistant strains continue to emerge and it is not unlikely that in near future, in the battle to overcome a bacteria, antibiotic era may prove to be only transitory. Further, antibiotic resistance is the problem of not only developing or poor countries or local clinical practice but of the whole world as there is increase in international travel. In turn, importance of infections have made the whole human population as a broth in a vial touched with contaminated loops.

Resistant Salmonellae are one of the major problems in clinical practice not only in India but elsewhere also. In developing countries where water supply, environmental sanitation and food hygiene are miserably suboptimal, typhoid and other salmonella (oro-faecal transmission) infections are of great threat to human life. The gravity of the problem can be conceived easily if we see the data of 1985 when 12.5 million cases of typhoid fever were noticed globally resulting in over 3,00,000 deaths that year. (Edelman and Levine, 1986).

Most cases of typhoid or enteric fever are caused by Salmonella enterica subsp . enterica serovar Typhi (S. Typhi) although. S. Paratyphi A and B and occasionally other serotypes are implicated. S. enteritidis and S. typhimurium are implicated mostly in food borne gastroenteritis, diarhoea, septicaemia etc. These isolates are frequently isolated in our laboratory from human as well as environmental sources.

It was observed in the routine practice that majority of these isolates were multidrug resistant. There is no report available on antibiotic resistance pattern of salmonellae not only from Bihar but also from the area lying between eastern India on this aspect. Therefore this vast area of Gangetic plain badly needs the antibiotic resistance pattern of salmonellae. River Ganga , in spite of vigorous efforts by different agencies still awaiting its due to be really clean, has yielded positive for salmonella strains.

Hence, the present study was planned to see the following:

1. Pattern of antibiotic resistance in S. Typhi
2. Level of drug resistance in different serovars.
3. Change in the level of drug resistance, if any, during the past two decades.

MATERIALS AND METHODS

[A] CHEMICALS AND SOURCE

Aesculin	: E.Merck, India
Agar Agar	:Hi Media, India
Arabinose	:Sigma, USA
Arginine hydrochloride:	Centron, India
Bromophenol Blue	:BDH Labs, England
Dipotassium hydrogen Phosphate	:Glaxo, India
Ferric ammonium citrate:	E. Merck, Germany
Glucose	:Glindia, India
Glycerol	:Ridel Chemicals, India
Hydrochloric Acid	:Glindia, India
Isoamly Alcohol	:Sarabhai Chemicals, India
Lysine hydrochloride	:Centron, India
Lactose	:Centron, India
Mannitol	:BDH, India
α-naphthol	:Ranbaxy, India
Ornithine hydrochloride	:Sisco, India
Peptone	:Hi Media, India.
Potassim dihydrogen Phosphate	:Glaxo, India
Potassium hydroxide	:BDH, India
Salicin	:BDH, India
Sodium citrate	:Glaxo, India
Sodium chloride	:Glaxo, India
Sodium hydroxide	:Glaxo, India
Sucrose	:Centron, India

[B] MEDIA

Mac-Conkey Agar	:Hi Media, Indian pH 7.2
Mueller Hinton Agar	:Hi Media, Indian pH 7.2
Nutrient Agar	:Hi Media, Indian pH 7.2
Nurtient Broth	:Hi Media, Indian pH 7.2
Bacteriological peptone	:Hi Media, Indian pH 7

Hi media Biphasic media

Hi media Triple Sugar Iron Media (TSI media) for salmonella

[C] BUFFERS

- Phosphate Buffer Saline (PBS, pH 7.2)

K_2HPO_4	1.21 gm
KH_2PO_4	0.34 gm
NaCI	8.0 gm
Distilled water	1.0 litre

- Phosphate Buffer Saline (PBS, pH 7.2)

NaCI	0.85 gm
Distilled water	100 ml

Sterilization of Media and Buffers:

All the media and buffers were sterilized at 15 lb/inch^2 pressure for 15 min except the glucose in minimal media, which was separately sterilized at 10 Ib/inch^2 pressure for 10 min.

D] ANTIBIOTIC DISCS

The following antibiotic discs were obtained from Hi Media and stored at 2-8ºC. The discs had the following concentration.

Ampicillin	Am	10
Amoxicillin	Au	10
Amikacin	Ak	30
Cephalexin	Cp	30
Cefotaxime	CTX	30
Ceftriaxone	CTR	30
Chloramphenicol	C25	
Ciprofloxacin	Cf	05
Cefuroxime	CEF`	30
Sparfloxacin	Sf	10
Furazolidone	Fz	50
Gentamicin	G	10
Trimethoprim	Tp	1.25
Tetracycline	T	10
Pefloxacin	Px	05
Ofloxacin	O	15
Netilmicin	N	30
Nalidixic acid	Na	30
Kanamycin	K	10
Norfloxacin	No	15
Streptomycin	St	15

[E] REFERENCE STRAINS		
Strain		Characteristics and use
1.	S Typhi	NCTC 10787 standard control for testing antibiotic resistance of all isolates

[I] BIOCHEMICAL CHARACTERIZATION OF THE STRAINS TO SPECIES LEVEL

100 isolates of Salmonella species were taken, purified and stocked in nutrient agar, slopes. The organisms maintained in nutrient agar slopes at room temperature, were sub-cultured when needed.

All strains were initially screened by using the following test:

1. Grams staining,
2. Motility by "hanging drop method" after incubation at 37ºC in BHI Broth.
3. Oxidase activity,
4. Glucose and Mannitol fermentation.

Only those strains that were Gram negative rods, motile, glucose and mannitol fermenting with production of acid and gas, citrate utilising and forming H_2S on triple sugar iron agar were considered belonging to the genus Salmonella.

For speciation, the following biochemical and related tests were performed by conventional methods as described by J.D. Sleigh and J.P. Duguid (1989).

1. Gas production from glucose, mannitol and sorbitol.
2. Fermentation of glucose, mannitol, arabinose, maltose dulcitol and sorbitol but not lactose, sucrose or adonitol.
3. Negative acetion production (V-P reaction) and a positive methyl red reaction.
4. Decarboxylation of lysine, ornithine and arginine but not glutamic acid.
5. Failure to produce indole, to hydrolyse urea or to deaminate phenylalanine. Citrate was utilised, malonate not utilised and gluconate not utilisied.
6. Urease was not produced.
7. No growth on KCN medium and gelatin not liquefied.
8. Hydrogen sulphide produced in ferrous chloride-gelatin medium.
9. Agglutination Test For Salmonella Tyhi Specific O9 Antigen using monoclonal Antisera for O9 Antigen (BD)

All the tests were performed at 37°C and the results were noted after 24 hours, unless otherwise indicated.

[II] ANTIMICROBIAL SUSCEPTBILITY AND RESISTANCE LEVEL

The test strains and the control strains of Salmonella typhi NCTC 10787 and were incubated over night in mueller hinton broth at 37°C. They were then diluted 1:1000 in PBS (pH 7.4) to a concentration of McFarland 5(10^4-10^5 viable cells/ml). The antimicrobial resistance was determined by Kirby Bauer's technique and minimum inhibitory concentration) (MIC) by plate dilution method (Stokes and Ridgeway 1980).

OBSERVATION

The present observations are based on bacteriological studies of 200 clinically suspected cases of typhoid fever patients at Patna Medical College and Hospital Patna.

Table No. – 1 Showing incidence of salmonellosis and other contaminant in present study of typhoid fever by blood culture.

S.N.	Name of organism	No. of organism isolates	Percentage (%)
1	S. Typhi	39	19.5
2	S. Paratyphi	03	01.5
3	Staph albus (epidermidis)	10	05.0
4	Sterile	148	74.0
	Total	200	100.0

It was obvious from the table No. 1 that, the incidence of Salmonella Typhi was 39 (19.5%), Salmonella paratyphi was 3 (1.5%), contaminant like steps. Albus was 10 (5%). Remaining culture were sterile 148 (74%). Incidence of contaminant such as Staphylococcus albus was excluded from the study.

Table No.- 2 Showing the incidence of S. Typhi and S. Paratyphi from 42 isolates.

S. No.	Organism	No. of isolates	Percentage
1	S. Typhi	39	92.8
2	S. Parathyphi	03	07.2
	Total	42	100.0

It was observed from table no. 2, that 39 (92.8%) isolates were of S. Typhi and 3 (7.2%) were of S. Paratyphi in 42 isolates.

Table No.-3 Showing the incidence of Widal test in suspected case of typhoid fever.

S. No.	Widal Test	Total no. of cases	Percentage (%)
1	Widal Positive with culture Positive	12	6
2	Widal Positive with culture Negative	123	61.5
3	Widal Negative with culture Negative	65	32.5
	Total	200	100.0

It was observed from table No. 3 that 67.5% (135) was widal positive in 200 fever cases.

Table No. 4 Showing the incidence of salmonellosis in different age groups.

S. No.	Age group	Total No. of case	% of total case	No. of salmonella Isolates	Percentage of salmonella	p-value
1	0-10	35	17.5%	4	2%	0.0103
2	11-20	50	25.0%	17	8.5%	
3	21-30	60	30.0%	16	8%	
4	31-40	35	17.5%	04	2.0%	
5	41-60	20	10%	01	0.5%	
	Total	200	100%	42	21.0%	

It was observed from the table no. 4 that 8.5% salmonellosis were found in age group 11-20 and 8.0% in the age group 21-30. The incidence salmonellosis was high in age group of 11-20 and slight less in the age group 21- 30, least in the age group above 40 years. The p-value was found to be 0.0103 which is significant.

Table no. 5 Showing incidence of salmonellosis in male and female sex.

S. No.	Age groups	Male	Positive case	p-value	Female	Positive case	p-value
1	0-10	18	3		17	1	
2	20-Nov	27	12		23	5	
3	21-30	41	13	0.028	19	3	0.079
4	31-40	23	3		42	1	
5	41-60	14	1		6	0	
	Total	123	32		77	10	
	Percentage	61.5	16		38.5	5	

Table no. 5 shows that the maximum number of cases affected were male i.e. 32 (16%), and affected cases were only 10 (5%), in case of female. It was observed that male and female ratio 3.2:1.

p-value for male patients was found to be 0.0280 which is significant whereas for female patients it is 0.079 which is not significant. The

overall p-value for positive cases in both sex was found to be 0.933 which is insignificant.

DISCUSSION

The present study has been under taken to know that, multidrug resistant Salmonella Typhi is becoming more prevalent in endemic countries. The patients infected with resistant strain of Salmonella have more severe illness, look toxic and have a higher incidence of Disseminated intravascular coagulation (DIC), hepatomegaly and three fold higher incidence of mortality due to ineffective oral antibiotic therapy, that leads to longer duration of illness.

The present discussion is based on drugs resistant of 200 clinically suspected cases of enteric fever. In those cases 35 were children (0-10 years) and rest were adult.

The study was conducted during the period from June 2017 to April 2020

INCIDENCE: -

Table No. 1 shows that 200 clinically suspected cases of enteric fever, there has been recovery of positive culture 42 (21% of total case studied). This result can be considered satisfactory, because febrile patients received treatment with different antibiotic by local quacks, before being admitted in the hospital. Approximately similar incidence (16.1%) was reported by DAS. U. et al in year 2000 in Ispat general hospital Rourkela. Rathore M.H et al in 1989 reported that isolation of salmonella by blood culture is 64% (104).

Table No 2 shows that isolates of Salmonella typhi and paratyphi is 39 (92.8%) and 3 (7.2%) respectively in present study. Approximately similar report made by Tran T. H. Et al in 1995, which showed S. typhi 2007(90.8%), paratyphi A 19 (8.3%) and S. Choleraesuis -2 (0.9%) in 228 positive salmonella isolates.

Table No. 3 shows that Widal test positive is 67.5% in 200 suspected cases of enteric fever 6% is widal positive with culture positive, 61.5% is widal positive with culture negative and 32.5% in widal negative with culture negative.

Rathore M.H. et al in 1989 had found that 73.5% widal test positive & Kabra S. K. Et in the year 2000 had found 75% of widal positive, which is approximately similar to the present studies.

AGE AND SEX INCIDENCE : -

Table No. 4 and 5 shows that most of the cases (55.5%) were between 11 to 30 years of age. This can be explained by the fact that this population group is highly active and mobile and takes part in maximum outdoor activity like agriculture and manual activities, which predispose these individuals to acquire infection from increased environmental exposure. The age incidence showed that salmonellosis is predominantly a problem of 11 to 30 years of age (16.5%), and more in the males of these age groups.

In the present study male febrile patients were 123 (61.5%), out of which 32 (16%) were culture positive. Female febrile patient were 77(38.5%), out of which only 10 (5%) cases were culture positive for salmonella.

Hence in the present study the male, female ratio of culture positive typhoid is 3.2:1. Approximately similar ratio of male cases to female case of typhoid was (3:2) has been reported by Rathore M.H. et al in 1989.

SENSITIVITY TO DRUG:

All the 42 strains of salmonellae isolates in the present study were found to be sensitive to various drugs (vide table no-4.8), in common use and resistant to other drugs, which is described below.

(1) Ceftriaxone, Sparfloxacine, Ofloxacine and Ciprofloxacine were found to 100% sensitive among isolated of salmonella strains in present study. Similar were observations of the Keusch G.T. et al in 1988, Rathore M.H. et al in 1989, Ishaque M et al in 1990, multidrug resistant isolates were 100% sensitive to ofloxacine, ceftriaxone. Chakravorty B.et al in 1993, Ciraj A.M. Et al in 1999, Kabra S.K. et al in 2000, were also found the same result.

Frost J.A. et al in 1996, DAS U. et al in 2000, Hirose K. et al in the year 2001, were found that few cases were resistant to ciprofloxacin, which may be due to consequence of indiscriminate use of these antibiotic, either singly or in combination.

(2) After ceftriaxone, sparfloxcin, ofloxacin and ciprofloxacin, the next group of antibiotics, which were sensitive to different isolates of salmonella strains, was cefotaxim, Cefuroxim and Pefloxacin. Similar was the observation of Rathore M.H et al in 1989, Ishque in et al in 1990. SAHA M.R et al 1992 and frost J.A et al in 1996.

(3) Cephalexine, Amoxicillin, Chloramphenicol were moderately against isolates of salmonella. Approximately similar were observation of Rowe B et al 1987. Deshmukh C T et al 1994. There is an increase in the sensitivity to these antibiotics, which have been rarely used over the past few years.

(4) Furazolidone, Norfloxacin, Nitilmicin, Kanamycin, Nalidixic acid, Amikacin, Gentamicin and Ampilcillin were found to be less sensitive against salmonella isolate of the present study. Approximately similar were observation of Jaynetra P et al in 1990 [resistant with many drug such as ampicillin (75%-92%).

(5) Streptomycin, Trimethoprim and Tetracyclin were found to be least sensitive against isolates of salmonella. Approximately similar were found to the least sensitive against isolates of salmonella. Approximately similar were observations by Cough T.D et al in 1983, Stur D et al in 1985, Keush G.T et al in 1988, Ishaque M. et al in 1990, Jayantra P. et al in 1990, Deshmukh C.T. et al in 1994.

However as compared to previous years there is increased incidence of resistance to Streptomycin, tetracycline, and Trimethoprim.

The most sensitive drugs for the treatment of salmonella infectioin were Ceftriaxone, Sparfloxacin, Ofloxacin and Ciprofloxacin. This was the observation of different study group's spreading all over the world. Present study also coroborate to this finding.

CONCLUSION

(1) Two hundred clinically suspected cases of typhoid fever were bacteriologically investigated. Salmonella were isolated from 42 (21%) of these cases.
(2) Salmonellosis was caused by salmonella typhi (92.8%) and S. Para typhi (7.2%).
(3) Maximum Number of isolation of salmonella was in 11-30 years age group.
(4) The incidence of salmonellosis was more common in male (16%) than in female (5%).
(5) People of the poor class were most sufferers of salmonellosis.

Other drugs such as cefotaxime, cefuroxime, Pefloxacin were 2nd most effective and sensitive. Moderately sensitive drugs against salmonella were chloramphenicol, Amoxicillin, and cephalexine.

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