Original Research Article

To Study The Prevalence and Pattern Of Resistance in Typhoid Fever Including Multi Drug Resistant Typhoid Fever (MDRTF) And Nalidixic Acid Resistant Salmonella Typhi (NARST) in a Tertiary Care Hospital

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Abstract

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. **Aim and Objectives:** To Study The prevalence and pattern of resistance in typhoid fever including MDRST and NARST. **Materials and Methods**: A Descriptive study / Case control study Were Conducted of All children admitted with clinical suspicion of typhoid fever and All children with fever (38oC and above) for atleast three days, with a laboratory confirmed positive culture of S.typhi.(WHO definition for confirmed cases of typhoid fever) 38. in All medical wards of Patna Medical College and Hospital, Patna (PMCH) From February 2020 to January 2021. **Results:** Incidence of multidrug resistant and quinolone resistant typhoid fever was found to be 43.6% and 20.5% respectively which vary with time and place. **Conclusion:** Hence there is a constant need to monitor antibiotic sensitivity pattern of S.typhi and periodically review the antibiotic policy in the hospital and the community so as to effectively utilize these antibiotics.

Key Words: Typhoid fever, MDRST, NARST

Introduction

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. On the basis of the literature 1, 2 and the incidence of typhoid fever recorded in control groups in large vaccine field trials with good laboratory support it has been estimated that approximately 17 million cases of typhoid fever and 600 000 associated deaths occur annually 3. The ratio of disease caused by S. typhi to that caused by S. paratyphi is about 10 to 1 in most of the countries where this matter has been studied. In areas of endemicity and in large outbreaks, most cases occur in persons aged between 3 and 19 years. Nevertheless, clinically apparent bacteraemic S. typhi infection in children aged under three years has been described in Bangladesh, India, Jordan, Nigeria, and elsewhere. Between 1% and 5% of patients with acute typhoid infection have been reported to become chronic carriers of the infection in the gall bladder, depending on age, sex and treatment regimen. The propensity to become a carrier follows the epidemiology of gall bladder disease, increasing with age and being greater in females than in males[1,2].

Typhoid fever is caused by Salmonella typhi, a Gram-negative bacterium. A very similar but often less severe disease is caused by Salmonella serotype paratyphi A. S. typhi can be identified in the laboratory by several biochemical and serological tests. One of the most specific serologic tests is that of polysaccharide capsule Vi, which is present in about 90% of all freshly isolated S. typhi and has a protective effect against the bactericidal action of the serum of infected patients. This capsule provides the basis for one of the commercially available vaccines. Vi antigen is present in some other bacteria (Citrobacter freundii, Salmonella paratyphi C and Salmonella dublin) but not in exactly the same genetic context[3,4].

Salmonellae can be characterized by their somatic (O) and flagellar (H) antigens, the latter existing in some serotypes in phases 1 and 2. Some salmonellae also have an envelop antigen called Vi (virulence). The O antigen is usually determined by means of the slide agglutination test with group-specific antiserum followed by agglutination with factor antiserum. H antigen is usually determined by means of the tube agglutination test.There are two categories of drug resistance: resistance to antibiotics such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole(MDR strains) and resistance to the fluoroquinolone drugs.Resistance to the fluoroquinolones may be total or partial.The so-called nalidixic-acid-resistant S. typhi (NARST) is a marker of reduced susceptibility to fluoroquinolones compared with nalidixic-acid-sensitive strains. Nalidixic acid itself is never used for the treatment of typhoid[5-7].These isolates are susceptible to fluoroquinolones in disc sensitivity testing according to current guidelines. However, the clinical response to treatment with fluoroquinolones of nalidixic-acid-resistant strains is significantly worse than with nalidixic-acid-senstive strains. There is a significant number of MDR strains from the Indian subcontinent and some other Asian countries (not Indonesia). S. typhi has recently emerged as a problem in Kenya. Nalidixic-acid-resistant strains are now endemic in many areas of Viet Nam and have also been reported from the Indian subcontinent and Tajikistan. There are disturbing recent reports of the emergence of fluorquinolone-resistant isolates in various parts of Asia and there have been a few reports of resistance to third-generation cephalopsorins in the same region. Reassuringly, however, many of these reports are coupled with evidence of the re-emergence of sensitive isolates in the same regions. The present problem is the emergence of quinolone resistance in addition to multi drug resistant salmonella typhi (MDRST). Multi Drug Resistance is defined as resistance to all the first line drugs namely ampicillin,

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chloramphenicol, and cotrimoxazole. Quinolone resistance is determined by sensitivity to nalidixic acid as suggested by WHO and these nalidixic acid resistant Salmonella typhi (NARST) implies Quinolone resistance to the clinician. the present scenario of emergence of nalidixic acid (Quinolone) resistant strains in addition to resistance to conventional drugs and wide variation in susceptibility of S.typhi among different geographical area, it is essential to find the resistance patterns in each locality and hospital including the MDR strains and NARST. In addition by comparing the clinical features of MDRTF with sensitive strains, the risk factor for MDR can be identified and this will provide a clinical clue to the presence of MDR and thus aid in formulating appropriate guidelines for therapy of typhoid fever[8,9].

Aim and Objectives: To Study The prevalence and pattern of resistance in typhoid fever including MDRST and NARST

Materials and Methods

A Descriptive study / Case control study Were Conducted of All children admitted with clinical suspicion of typhoid fever and All children with fever (38oC and above) for atleast three days, with a laboratory confirmed positive culture of S.typhi.(WHO definition for confirmed cases of typhoid fever) in All medical wards of Patna Medical College and Hospital, Patna (PMCH) From February 2020 to January 2021.

Exclusion criteria: Associated infections (mixed infections) like UTI, malaria, leptospirosis, prior antibiotic usage.

Suspected cases of typhoid fever were admitted in our hospital and detailed history was taken including age, sex, duration of fever and associated symptoms.Also history of previous treatment, socio-economic status and typhoid immunization was elicited. Then complete physical examination was performed to assess the general condition of the patient, nutritional status and temperature; and examination of systems was done. Significant hepatomegaly was considered to be present, only when the liver size is more than 3.5 cms in new born and 2 cms in children. After that blood was drawn for investigations like Widal test, enteric culture; and also for other investigations to rule out other causes of fever (like smear for malarial parasite, MSAT, non-enteric culture). Urine routine examination and culture was done. Blood for enteric culture was taken in Brain-Heart infusion (BHI) broth. One ml of blood was taken using sterile syringe / scalp vein set and put into 10ml of BHI broth to give an optimal dilution of 1:10. Since blood contains substances that inhibit the growth of bacilli, it is essential that the broth be taken in sufficient quantity to provide at least four fold dilution of blood[10,11]. Liquoid (Sodium polyethanol sulphonate) was added to BHI broth during its preparation to counteract the bactericidal action of blood. BHI broth was kept in refrigerator prior to use to avoid bacterial contamination. Before adding blood, the BHI broth was brought down to normal temperature and blood was added. After adding blood, BHI broth was immediately transported to microbiology lab of the hospital. If it was not possible, as in night times, the bottle was kept in the incubator and then transported to lab. This avoided bacterial overgrowth and enhanced the chances of positivity of salmonella typhi growth. In the lab, BHI broth was incubated at 37^{O}C in the incubator for 24hrs. Then subculture was done in MacConkey agar and salmonella shigella agar, which is a special media for S.typhi. After overnight incubation at 37OC, the pale smooth, nonlactose fermenting colonies were looked for. If present, then battery of tests was done to confirm S.typhi growth. After confirming the growth, antibiotic sensitivity was done in Muller-Hinton agar, using Kirby-Baur technique.

Statistical Analysis

1. Proportion of MDRTF and NARST among culture proven typhoid fever was found.
2. To associate various features to drug resistance, univariate analysis was done to arrive
at odds ratio (OR) with 95% confidence interval.
3. To associate how far individual factors contribute independently for drug resistance,multi variate analysis was done.

Results

Incidence of multidrug resistant and quinolone resistant typhoid fever was found to be 43.6% and 20.5% respectively which vary with time and place. Hence there is a constant need to monitor antibiotic sensitivity pattern of S.typhi and periodically review the antibiotic policy in the hospital and the community so as to effectively utilize these antibiotics. Majority of S.typhi isolates were found to be resistant to cotrimoxazole (74.4 %) and ampicillin (59 %) and most strains were observed to be sensitive to ceftriaxone (89.7%) and amikacin (87.2%). Though amikacin was found to be sensitive in as high as 87.2%,its usefulness in vivo need to assessed.

Chloramphenicol sensitivity was observed among 56.4% of the isolates. More importantly some of the quinolone resistant and ceftriaxone resistant strains were found to be sensitive to chloramphenicol. Age less than five years, prolonged fever for more than seven days, hepatosplenomegaly, abnormal liver function test and thrombocytopenia are specific risk factors for multidrug resistant typhoid fever and need early and aggressive management for prevention of complications. Quinolone resistance may be a problem in future in typhoid fever and specific risk factors and alternative therapeutic strategies need to be evaluated[12-16].

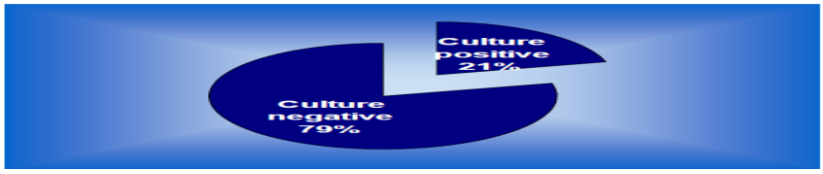

Fig 1:Culture

Table 1 : Antibiotic susceptibility pattern among 39 culture positive cases

S.no.	Drug	Sensitive		Resistant	
		N=	%	N=	%
1	Amicillin	16	41	23	59
2	Chloramphenicol	22	56.5	17	43.6
3	Cotrimoxazole	10	25.6	29	74.4
4	Cefotaxime	30	76.5	9	23.1
5	Ceftriaxone	35	89.7	4	10.3
6	Nalidixic acid	31	79.4	8	20.6
7	Ciprofloxacin	31	79.4	8	20.6
8	Amikacin	34	87.2	5	12.8

Discussion

There is a wide variation in the culture positivity rate reported from different studies in India. It is as low as 12% in a recent large study conducted in JIPMER to as high as 80% in some studies. The culture positivity rate from the present study is 21%. This is quite an improvement compared to the culture positivity rates from the same institute in the preceeding years. It was seen that the majority of the strains grown were found to be resistant to cotrimoxazole (74.4%) and ampicillin (59%) and most strains were found to be sensitive to ceftriaxone (89.7%) and amikacin (87.2%). Though amikacin is not considered to be a useful drug against Salmonella typhi, it has shown a very high sensitivity in the invitro susceptibility testing. Whether it is really useful in vivo either as monotherapy or as a combination especially in drug resistant cases needs to be determined. Majority of the strains were also sensitive to quinolones (79.4%) and cefotaxime (76.9%). Though there is a wide variation in chloramphenicol sensitivity reported from the different parts of the country, ranging from 0% sensitivity to 90% sensitivity 24,25, in the present study it is observed that chloramphenicol is sensitive in 56.4%. More importantly, chloramphenicol has been found to be quite sensitive in some of the quinolone resistant and cephalosporin resistant isolates. Thus, as reported in some of the studies, chloramphenicol sensitivity may be re-emerging and there is a constant need to monitor the sensitivity pattern of S.typhi isolates.Among the 39 cultures grown, the incidence of multi-drug resistance was found to be 43.7% (n = 17). Among the 17 multi-drug resistant strains in the present study, 15 were sensitive to ceftriaxone and 14 were sensitive to quinolone. Comparing other studies from India, the incidence of multi-drug resistant typhoid fever varied from 10% to 93%.The incidence of quinolone resistance in the present study was found to be 20.5% (n = 8). Though there are number of studies from India reporting the incidence of multi-drug resistant typhoid fever, the data on quinolone resistance is very limited 23,24,30,33. Among the eight quinolone resistant cases in the present study, six were sensitive to ceftriaxone and five were sensitive to chloramphenicol. Among the two cases which were quinolone and ceftriaxone resistant, one was sensitive to chloramphenicol and the other resistant to all the other drugs, except amikacin and ceftazidime.

Clinical Profile

Age: The mean age of the patients among the entire study group was 5.4 ± 2.6 years, while the mean age in the drug resistant group and the drug sensitive group were 4.0 ± 2.1 and 6.4 ± 2.5 respectively. Though the total number of children < 5 years was 19 in the overall study group, majority (70.6%, n = 12) were in the drug resistant group and only 31.8% (n = 7) were in the drug sensitive group (P = 0.02).

Sex: There were totally twenty two males and seventeen females in the study group. There was no difference in the sex distribution among drug resistant and sensitive groups. There were no seasonal variation and cases occurred throughout the year indicating that typhoid fever is endemic in this region. The mean duration of fever in the whole group was 6.9 ± 2.4 days, while in the drug resistant group it was 8.5 ± 1.9 days and it was 5.7 ± 2.0 in the drug sensitive group (P = 0.001). Though fever was the presenting problem in all the children, prolonged fever > 7 days was found in significantly major proportion in the drug resistant group (76.5%, n = 13) than in the drug sensitive group (31.8%, n = 7). Thus prolonged fever > 7 days was identified to be one of the important risk factor for drug resistance.Though respiratory symptoms like running nose, sneezing, cough etc were found more in the drug resistant group (70.6% vs 36.4%), it did not meet the required statistical criteria (P > 0.05). Chills and rigor was found in one-third, abdominal symptoms like nausea, vomiting and loose stools were found in nearly half of the patients and central nervous symptoms like head-ache and letharginess were found in nearly 10% of the patients, without any difference between the two groups. There were no cases of profound loss of consciousness or seizures.Toxic look was found in 11 out of the 39 patients and coated tongue was found in 12 patients without much difference between the two groups. Isolated hepatomegaly was found in six out of the 39 children, four in the multi-drug resistant group and two in the drug sensitive group. Whereas isolated hepatomegaly was found only in few patients, isolated splenomegaly was found almost exclusive in the drug sensitive group with only one patient from drug resistant group had isolated splenomegaly. Hepatosplenomegaly was found in significantly major proportion in drug resistant group (70.6%, n =12), while in the drug sensitive group it was only 18.2% (n=4) (P <0.001). It is also interesting to note that all the patients had one or other form of organomegaly, with isolated splenomegaly predominating in the drug sensitive group and hepatosplenomegaly in the resistant group. Lung signs occurred in almost one-fourth of the patients, though equally in both the groups. Though higher complication rates have been observed in some of the studies in India 21,22,32, complications are few in the present study. Abnormal liver function test and thrombocytopenia were the two major complications observed, primarily in the multi-drug resistant group and both assuming a statistically significance. Though no cases of overt clinical jaundice was observed, abnormal liver function in the form of mild elevation of bilirubin or more than twice the normal level of liver enzymes were observed in eight cases, seven in the multi-drug resistant group (41.2%) and one from sensitive group (4.5%) (P <0.01). Similarly thrombocytopenia, defined by platelet count <100,000, were found in nine patients, significantly more in the drug resistant group (41.2%, n = 7), while two patients from drug sensitive group (9.1%) also had thrombocytopenia. Except for the one patient, which presented with malena, none of the other had any clinical bleeding. Two of the patients, both from the multi-drug resistant group, had shock at presentation. Both the children were corrected with two boluses of isotonic fluids. The exact reason for shock, whether it is fluid leak into third space, inadequate intake of oral fluids or increased loss of fluids secondary to high grade fever or combination of these, were not known. But both the children recovered quickly without any other complications . Only one patient from the multi-drug resistant group had gastro-intestinal hemorrhage in the form of malena. The child also had a lowest platelet count of 85,000. The reason for the hemorrhage, whether due to thrombocytopenia or intestinal foci of salmonella was not known. The child had fever lasting for more than six days after admission, but recovered subsequently.

Conclusion

Incidence of multidrug resistant and quinolone resistant typhoid fever was found to be 43.6% and 20.5% respectively which vary with time and place. Hence there is a constant need to monitor antibiotic sensitivity pattern of S.typhi and periodically review the antibiotic policy in the hospital and the community so as to effectively utilize these antibiotics.Majority of S.typhi isolates were found to be resistant to cotrimoxazole (74.4 %) and ampicillin (59 %) and most strains were observed to be sensitive to ceftriaxone (89.7%) and amikacin (87.2%). Though amikacin was found to be sensitive in as high as 87.2%,its usefulness in vivo need to assessed. Chloramphenicol sensitivity was observed among 56.4% of the isolates.More importantly some of the quinolone resistant and ceftriaxone resistant strains were found to be sensitive to chloramphenicol. Age less than five years, prolonged fever for more than seven days, hepatosplenomegaly, abnormal liver function test and thrombocytopenia are specific risk factors for multidrug resistant typhoid fever and need early and aggressive management for prevention of complications. Quinolone resistance may be a problem in future in typhoid fever and specific risk factors and alternative therapeutic strategies need to be evaluated.

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