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EMERGING RESISTANCE;
ANTIBIOGRAMS OF SALMONELLA STRAINS

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ABSTRACT… **Objectives:** To study the bacteriological profile of enteric fever and their antibiotic sensitivity pattern to commonly used antibiotics in view of emerging resistance at Civil Hospital Karachi (C.H.K.) and provide a guideline for making a protocol for empirical antibiotic therapy where culture facilities are not available. **Methodology: Design:** Descriptive laboratory Based study. **Place and Duration of study:** The study was conducted from 1st January, 2010 till 30th June, 2012 at the Central Lab, Civil Hospital Karachi. **Patients and Methods:** A cross sectional analysis was done on a total of 37,805 blood specimens sent for C/S from the entire inpatient and outpatient departments to the central lab, CHK. All blood cultures were inoculated in thioglycolate broth and incubated at 37° for 7 days. Positive blood cultures were processed, colonies were identified using standard biochemical tests and antibiotic susceptibility was checked by Kirby-Bauer disc diffusion method as per CLSI criteria. **Results:** Out of these specimens, 430 samples were found positive for the salmonella strains, among them 395 (91.86%) were Salmonella typhi and 35 (8.14%) were Salmonella paratyphi A. Salmonella infection was more common in male (55.85%) and more prevalent in children under 10 years of age (34.88%). The resistance pattern for Amoxicillin, Chloramphenicol, Co-trimoxazole, was (29%), (41%) and (5%) respectively; while the resistance was found to be higher in the commonly prescribed drugs belonging to first and second generation of cephalosporins and flouroquinolones. **Conclusion:** Amoxicillin and Chloramphenicol, the first line of drugs for the treatment of enteric fever are losing their efficacy and most of the organisms have developed resistance. Also, resistance against the second line of therapy, involving the use of cephalosporins and quinolones is rapidly emerging.

Key words: Enteric fever, Salmonella typhi, Antibiotic Susceptibility Pattern, Pakistan

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INTRODUCTION

Typhoid fever or enteric fever has remained in great concerns for mankind since many decades, and had frequently shown outbreak patterns in different regions of the world. WHO survey estimates the global incidence of typhoid fever at 0.3%.[1] While in other studies, the worldwide incidence is estimated to be 16 million cases out of which 7 million had been reported in South Asia alone, with a mortality estimation reaching to 600,000 annually.[2] With the improvement in sanitation, food production and water treatment facilities in western world, typhoid fever has become rare in such parts; however, third world countries especially Asian urban regions are still being considered as endemic.[3] The prevalence of typhoid fever in Pakistan is hard to predict as there had been no significant data over it although WHO states that the overall incidence of typhoid fever is approximately 412 cases per 100,000 per year[4], making it to the 4th most common cause of death in Pakistan.[5]

In 1982, Dr. William Osler defined typhoid as "an infectious disease, characterized anatomically by hyperplasia and ulceration of the lymph follicles of the intestines, swelling of the mesenteric glands and spleen, and parenchyma changes in other organs. The bacillus of Eberth now named S. typhi is constantly present in the lesion. Clinically the disease is marked by fever, a rose-colored eruption, diarrhea, abdominal tenderness and enlargement of the spleen, but these symptoms are extremely inconstant and even the fever

varies in its character", and till now this exact definition hasn't changed much.[6] The disease is predominantly a school disease effecting mainly children and young adults. The main symptoms are fever, abdominal pain, vomiting, and headache. Diarrhea, loss of appetite and in some cases hepatomegaly and splenomegaly are also observed.[5] Salmonella species, belonging to family Enterobacteriaceae are the main causative agents of enteric fever, the most common of which are S. typhi followed by S. paratyphi A, B and C.[7] The organism commonly enters via oral route through contaminated food, water and shows a wide variety of host like birds, chicken, fish, poultry and even humans.

The first major drug resistance in salmonella species was reported in Pakistan in 1987[8], and the continuous change in its resistance and decrease susceptibility to first line agents i.e. Ampicillin, Chloramphenicol and Trimethoprim-sulphamethoxazole (TMP-SMX), the disease has significantly altered the presentation, pattern and therapeutic strategies.[8] Chloramphenicol had been considered as a gold standard therapy for typhoid. However, studies from 1967 to 1982 illustrates that the sensitivity pattern of Salmonella typhi was Ampicillin, Cotrimoxazole and Chloramphenicol.[9] During 1978, it was observed that from the samples of S. typhi, only 19% was sensitive to Ampicillin, 59% sensitive to Cotrimoxazole and 4% sensitive to Chloramphenicol.[9] In Pakistan during September 2004, a study revealed a substantial number of isolates (35-37%) were resistant to first line drugs, however resistance was low against Cephalosporins (up to 2.6%), and Fluoroquinolones (1.2%).[10]

Many reports from developing countries show that the clinical presentation, diagnosis and treatment of enteric fever have significantly changed; therefore, antibiotic susceptibility of these Salmonella strains requires reassessment from time to time. We designed this study to study the bacteriological profile of enteric fever and their antibiotic sensitivity pattern to commonly used antibiotics in view of emerging resistance at Civil Hospital Karachi (C.H.K.) and provide a guideline for making a protocol for empirical antibiotic therapy where culture facilities are not available.

METHODS

This was a descriptive laboratory based study. The study was undertaken in the central lab, Civil Hospital Karachi over the period of two and a half years (from 1st January, 2010 till 30th June, 2012). Blood specimens from all age-groups with minimum age of one month and both sexes for culture and sensitivity were taken from all the inpatient and outpatient department of CHK. All blood cultures were inoculated in thioglycolate broth and incubated at 37°C for at least 72 hours. Positive blood cultures were processed, colonies were identified using standard biochemical tests and antibiotic susceptibility was checked by Kirby-Bauer disc diffusion methods as per CLSI criteria.[25] All the blood culture and sensitivity reports showing positive cultures for Salmonella typhi and Salmonella paratyphi A, B and C were included in the study. The sensitivity pattern of all positive blood cultures were recorded for further analysis. The analysis was done using SPSSv16.0.

All the Salmonella strains were tested against the drugs outlined in the results. The complete sensitivity, intermediate sensitivity or resistance was judged on the basis of measurement of zone of minimum growth inhibition around individual disc.

RESULTS

A total of 37,805 specimens were received for blood culture during the study period from the inpatients and outpatients departments of Civil Hospital Karachi; out of which 430 (1.14%) samples were found positive for Salmonella strains. The strains of Salmonella which were identified in these samples included Salmonella typhi (n=395, 91.86%) and Salmonella paratyphi A (n=35, 8.14%). No strains of Salmonella paratyphi B and C were identified in these samples. The relative proportion of the two Salmonella strains identified is shown in Figure-1.

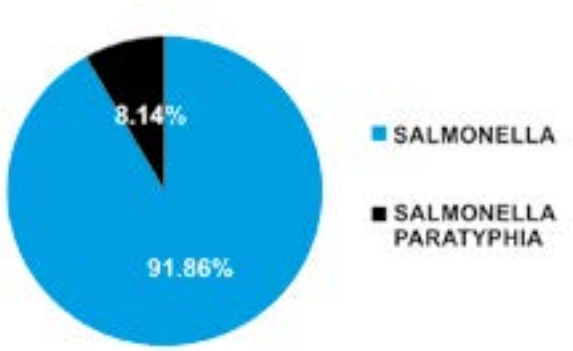

Figure-1. Shows the relative proportion of S.Typhi and S.Paratyphi A

There were 240 males (55.85%) and 190 females (44.18%) in the study with the male to female ratio of 1.26. Infection was found to be more prevalent in children with one third of cases (n=150, 34.88%) in the age group of 1-10. (The minimum age included in the study was one month. to be deleted as mentioned in methodology)

The results for the antibiotic susceptibility are shown in Table-I. In the Penicillin group, 70% of the strains were found sensitive to Amoxicillin while only 60% of the strains were sensitive for Amoxicillin + Clavulanic acid.

ANTIBIOTIC GROUP	ANTIBIOTIC	RESISTANT R		INTERMEDIATE I		SENSITIVE S	
		n	%	n	%	n	%
CARBAPENEM	IMIPENEM	90	20.9	15	3.4	325	75.5
AMINOGLYCOSIDES	AMIKACIN	15	3.4	5	1.2	410	95.3
	GENTAMICIN	15	5.8	20	4.6	385	89.5
PENICILLIN	AMOXYCILLIN	125	29.1	5	1.2	300	69.7
	AMOXYCILLIN+CLAVULANIC ACID	170	39.5	0	0	260	60.4
TETRACYCLINE	DOXYCYCLINE	10	2.3	0	0	420	97.6
CEPHALOSPORIN (1ST)	CEPHALEXIN	75	17.4	0	0	355	82.5
CEPHALOSPORIN (2ND)	CEFACLOR	70	16.2	5	1.2	355	82.5
	CEFUROXIME	60	13.9	5	1.2	365	84.8
	CEPHRADINE	75	17.4	0	0	355	82.5
CEPHALOSPORIN (3RD)	CEFIXIME	165	38.3	10	2.3	265	61.6
	CEFTAZIDIME	50	11.6	5	1.2	375	87.2
	CEFOTAXIME	170	39.5	10	2.3	260	60.4
	CEFTIZOXIME	110	35.5	10	2.3	310	72.1
	CEFTRIAXONE	85	19.7	15	3.4	330	76.7
CEPHALOSPORIN (4TH)	CEFIPIME	15	3.4	0	0	415	96.5
FLOUROQUINOLONES (1ST)	NALIDIXIC ACID	140	32.5	20	4.6	270	62.8
FLOUROQUINOLONES (2ND)	CIPROFLOXACIN	100	23.2	5	1.2	325	75.5
	NORFLOXACIN	100	23.2	15	3.4	315	73.2
	OFLOXACIN	85	19.7	20	4.6	325	75.5
	ENOXACIN	35	8.1	5	1.2	390	90.6
	SPARFLOXACIN	25	5.8	5	1.2	400	93.1
FLOUROQUINOLONES (3RD)	LEVOFLOXACIN	35	8.1	5	1.2	390	90.7
FLOUROQUINOLONES (4TH)	MOXIFLOXACIN	10	2.3	0	0	420	97.6
	TMP-SMX	20	4.6	0	0	410	95.3
	CHLORAMPHENICOL	175	40.6	10	2.3	195	56.9
	VANCOMYCIN	0	0	0	0	430	100
	CEFOPERZONE/SULBACTAM	15	3.4	0	0	415	96.5
	PIPERACILLIN/TAZOBACTAM	5	1.2	10	2.3	415	96.5
MONOBACTAM	AZTREONAM	5	1.2	0	0	425	98.8

Table-I. Shows Antibiotic Susceptibility pattern of patients with S.Typhi and S.Paratyphi A (n=430)

In the Cephalosporins group, Cefipime was found to be most sensitive while low levels of sensitivities were found for Cefixime and Cefotaxime (62% and 60%, respectively) which are the commonly prescribed drugs in this antimicrobial group. In the Flouroquinolones group, lowest rate of sensitivity was found in Nalidixic acid while the 3rd and 4th generation Quinolones had the highest level of sensitivity for Salmonella strains.

Out of the usually prescribed drugs for the treatment of enteric fever, Chloramphenicol had the highest level of resistant Salmonella strains (40.6%). The antibiograms of all the generations of Cephalosporins and Flouroquinolones is depicted in figure-2 and 3.

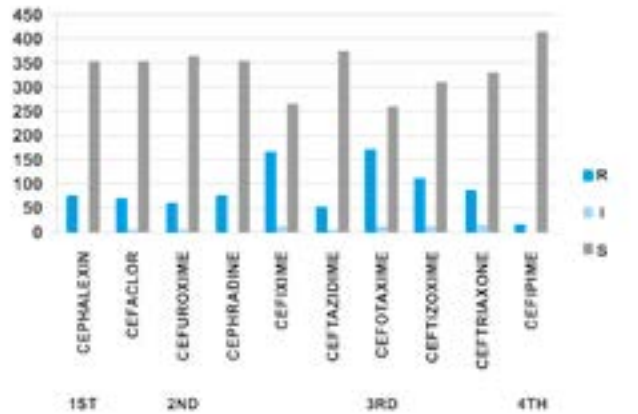

Figure-2. Antibiogram of S.Typhi and S.Paratyphi A in relation to various drugs of Cephalosporin group (n=430)
*(Susceptibility Pattern: R=resistant, I=intermediate, S=sensitive)

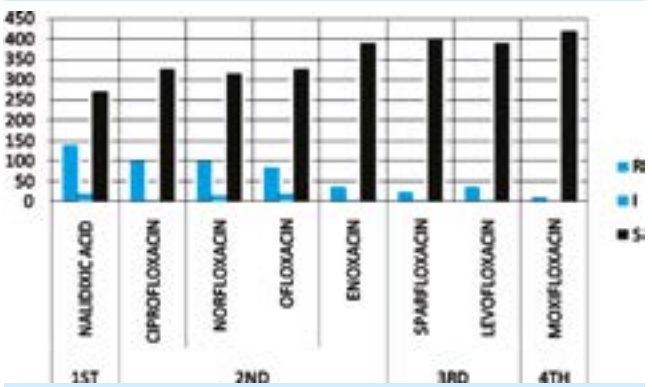

Figure 3: Antibiogram of S.Typhi and S.Paratyphi A in relation to various drugs of Flouroquinolone group (n=430)
*(Susceptibility Pattern: R=resistant, I=intermediate, S=sensitive)

DISCUSSION

Typhoid fever remains a major public health problem and endemic in developing countries like Pakistan. Poor sanitation, unhygienic conditions and; lack of personal and communal hygiene aid to spread of the disease.[8] It has a higher incidence in children below age of sixteen[11], threatening infant, pediatric and teenage population in our country. Our study found it prevalent in children with one third of cases in under 10 years of age, mostly in males, similar to the studies that had been conducted in previous years.[5,8]

Blood culture was the source of the isolates, Salmonella (enterica to be deleted) typhi and paratyphi A strains were the two isolates 91.8% and 8.14% respectively. These servers are restricted to humans and can cause systemic diseases. Salmonella paratyphi A has genomic similarity to Salmonella typhi however it is more recent evolutionary strain.[12] This finding is suggestive that our population is more exposed to Salmonella typhi strain than Salmonella paratyphi results similar in line to already conducted studies in the world.[9,13,14]

Chloramphenicol has been gold standard drugs and first line therapy in the treatment of typhoid fever globally, but due to indiscriminate use of the drug, Increase in its resistance level has been observed[15], approximately 41% samples in our study were also found resistant to the drug. Alternate drugs Amoxicillin, Amoxicillin + Clavulanic acid and Co Trimaxazole were also suggestive of resistance.[16,17] They shows an average susceptibility profile of 70%, 60% and 95% respectively according to our study decreasing their efficacy.

Multi drug resistant Salmonella typhi (MDRST) is a frequent problem encountered by physicians in the developing world, hence Flouroquinolones and Cephalosporins have gained attention.[18]

Cephalosporins have a good efficacy, while some resistance is found in commonly prescribed 1st/2nd generation still 3rd generation Cephalosporins are better proved[19], Cefexime 62%, Cefotaxime

60 % sensitive and both effective.[20] Our results are more optimistic for Cefipime, 4th generation Cephalosporin with 96.5 % sensitivity. Parenteral 3rd generation are commonly prescribed for typhoid fever[14] though they stand inferior to Quinolones according to other studies.[21]

Quinolones has now emerged drug of choice for improved treatment, they decrease hospitalization and are short term regimen.[22] Nalidixic acid is an important marker for treatment failure of Ciprofloxacin,[23] Nalidixic acid resistance is very common in isolates of S. typhi and is associated with reduced susceptibility of Flouroquinolones[24], we found our cultures sensitive to Ciprofloxacin, Norfloxacin, Ofloxacin in 75%, 73% and 75% of the samples respectively and lowest sensitivity to Nalidixic Acid. However 3rd generation Levofloxacin and 4th generation Moxifloxacin were found to be the most sensitive Quinolones, with the sensitivity of 90.7% and 97.6% respectively, making newer generations 1st choice in the group.[7]

Drug over use and inappropriate prescription are main reasons for the emerging resistance in Salmonella typhi strains and treatment failure, increasing morbidity and mortality of the disease. We suggest that organism susceptibility to antibiotics should be rationalized to check this problem and provide a better treatment regime for the patients.

CONCLUSION

Amoxicillin and Chloramphenicol, the first line of drugs for the treatment of enteric fever are losing their efficacy and most of the organisms have developed resistance. Also, resistance against the second line of therapy, involving the use of Cephalosporins and Quinolones is rapidly emerging. However, most of the cases of enteric fever can be successfully treated with the help of 3rd and 4th generation Cephalosporins and Quinolones.

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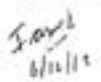
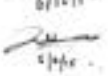
2	Dr. Usama Khalid Choudry	Study design, Data acquisition, Analysis, Manuscript drafting	
3	Dr. Iram Saddiqa Aamir	Critical review of the article	
4	Dr. Qurrat-ul-Ain Aqeel	Data interpretation, proof reading, manuscript drafting	
5	Dr. Syed Ahsan Uddin Ahmed	Study design, data acquisition, Analysis, Manuscript drafting	
6	Dr. Ghulam Fatima	Data interpretataion