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Emergence of Multi-drug Resistant Strains of *Salmonella typhi* and *Paratyphi A* in the Rawalpindi/Islamabad

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To address the increasing incidence of resistant strains of *Salmonella typhi* and *Salmonella paratyphi*, this study was carried out determine the efficacy of different antibiotics against these isolates and to ascertain the existence of multi-drug resistant strains, specifically in the Rawalpindi/Islamabad area. The antibiogram pattern of a total of 22 *Salmonella* strains, 14 *Salmonella typhi* and 8 *Salmonella paratyphi A* was determined using the disc diffusion method. The *Salmonella* isolates were identified through microscopy and biochemical test procedures and were confirmed up to species level by serotyping using the slide agglutination procedure. A total of 22 *Salmonella* isolates were subjected to antimicrobial sensitivity tests. Of these, 14 (63.6%) were serologically identified as *Salmonella typhi* and 8 (36.4%) as *Salmonella paratyphi A*. Out of the 14 *Salmonella typhi* strains, all of them were found to be sensitive to Amikacin, Ceftraxone, Ciprofloxacin, Enoxacin and Ofloxacin, 11 (78.5%) were also sensitive to Cefazolin/Cefradine, Ceftazidime, Cefotaxime and Gentamicin, while 7 (50%) of the strains were sensitive to Chloramphenicol. Ten strains of the *Salmonella typhi* strains were determined to be resistant to Ampicillin and Co-trimoxazole, 9 (64.2%) resistant to Tetracycline and 6 (42.8%) resistant to Chloramphenicol. Ten strains were resistant to a combination of Ampicillin plus Co-trimoxazole, 9 (71.42%) resistant to Ampicillin plus Tetracycline and Ampicillin plus Chloramphenicol and 6 (42.8%) of the strains were resistant to Co-trimoxazole plus Tetracycline combination. Seven (87.5%) strains of *Salmonella paratyphi A* were sensitive to Ofloxacin and Ceftazidime, while 6 (75%) were determined to be sensitive to Cefotaxime, Enoxacin and Gentamicin. Five (62.5%) of the isolates were found to be resistant to Co-trimoxazole and 4 (50%) resistant to Ampicillin, Cefazolin/Cephradine and Tetracycline. Four (50%) of the *Salmonella paratyphi A* isolates showed resistivity to a combination of Co-trimoxazole plus Ampicillin, Co-trimoxazole plus Tetracycline and Co-trimoxazole and Cefazolin. With the alarming increase in multi-drug resistant *Salmonella typhi* and *paratyphi A* to conventional antibiotics, typhoid fever must be treated very cautiously and newer antibiotics must only be used when they are indicated so as to prevent the further development of microbial resistance against them.

Key words: Multi-drug resistant, *Salmonella typhi*, *Salmonella paratyphi A*

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INTRODUCTION

Salmonella food poisoning is usually a self-limited disease. In general, these infections do not respond well to treatment with antibiotics and a persistent carrier state commonly follows infection. The incidence of salmonellosis is greater during the summer months, which is probably due to the ambient temperatures conducive for proliferation of *Salmonella* sp. in un-refrigerated food. Humans acquire the infection by ingesting the organisms in contaminated animal products, foods or water, whereas other *Salmonella* species are found only in humans with infections transmitted by human carriers[1]. Environmental sources of *Salmonella* sp. include water, soil factory, kitchen, animal feces, raw milk and meat. All age groups are susceptible to salmonellosis, but it is more severe in infants and the elderly[2].

Salmonella sp. are hardy microorganisms that can survive in moist environments and as well as in frozen state for several months. *Salmonella* are also able to tolerate hostile conditions such as low gastric pH and the antimicrobial actions of peptides secreted by the enterocytes[3].

Antibiotics used in acute as well as in chronic salmonellosis, belong to different chemical groups and remain the sole remedy for this infection. However, development of resistance to an array of antibiotic groups has been reported, which may be attributed to various factors. These include under-dosage, the cost associated with the lengthy duration of therapy and the exposure time of the bacterium to long-acting antimicrobial with short half lives, global spread of the causative agent over short periods of time, facilitated by excessive traveling and reduced travel time, as well as mass human migration resulting from famine, poverty and environmental or natural devastation, as well as the indiscriminate use of antibiotics, particularly in developing countries[4,5].

Since 1989 outbreaks caused by *Salmonella typhi* strains resistant to Chloramphenicol, Ampicillin and trimethoprim, with additional resistance to streptomycin, sulphonamide and Tetracycline have been reported in many developing countries, particularly in Pakistan and India[6]. Multi-resistant strains have also caused outbreaks in Bangladesh and in several southeast Asian and throughout Africa[7].

In Pakistan, *Salmonella typhi* strains sensitive to Ciprofloxacin, Oflaxacin and Enoxacin have been reported[8]. In Rawalpindi, studies have documented that the improper use of quinolones, specifically, may however lead to the impending menace of resistance among typhoid *Salmonella*[9].

The continuous increase in antibiotics resistance among *Salmonella* poses a serious problem, particularly with regard to the treatment of typhoid fever and systemic infections caused by other *Salmonella* serotypes[10]. Furthermore, as antibiotics remain the major source of treatment to minimize the severity of salmonellosis, the continuous use, particularly unmonitored use, of these antibiotics may lead to the emergence of resistant strains. Besides the routine activities to control typhoid fever, an accurate and continuous surveillance is necessary to facilitate the quick identification of multidrug-resistant *Salmonella* strains and prevent their spread.

To address the increasing incidence of resistant strains of *Salmonella typhi* and *Salmonella paratyphi*, this study was carried out to determine the efficacy of different antibiotics against these isolates and to ascertain the existence of multi-drug resistant strains, specifically in the Rawalpindi/Islamabad area.

MATERIALS AND METHODS

The antibiogram pattern of a total of 22 *Salmonella* strains, 14 *Salmonella typhi* and 8 *Salmonella paratyphi* A, isolated from patients diagnosed with gastroenteritis, typhoid fever and bacteremia over a period of one year, from August 2000 to July 2001, was determined using the disc diffusion method.

The antimicrobial test procedures were carried out at the Bacteriology Laboratory of the Public Health Division at the National Institute of Health, Islamabad. The *Salmonella* isolates were identified through microscopy and biochemical test procedures and were confirmed up to species level by serotyping using the slide agglutination procedure[11].

The antimicrobial sensitivity test of the identified *Salmonella* strains was carried out by the diffusion disk method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Muler Hinton agar plates were used for antimicrobial testing. Colonies were mixed in peptone water, matched the turbidity of suspension with standard 0.5% barium chloride solution. The entire medium of Muller Hinton plate was swabbed by picking bacteria from suspension and disks of various antibiotics were placed on the labeled agar plates with the help of sterile forceps. After 24 h incubation at 37°C these plates were examined, diameter of zone of inhibition measured to ascertain the sensitivity and resistant pattern to the particular antibiotic[11]. *E. coli* ATCC 25922 was included for quality control.

RESULTS

A total of 22 *Salmonella* isolates were subjected to antimicrobial sensitivity tests. Of these, 14 (63.6%) were serologically identified as *Salmonella typhi* and 8 (36.4%) as *Salmonella paratyphi A*.

Out of the 14 *Salmonella typhi* strains, all of them were found to be sensitive to Amikacin, Ceftraxone, Ciprofloxacin, Enoxacin and Ofloxacin. In addition, 11 (78.5%) of these strains were also sensitive to Cefazolin/Cefradine, Ceftazidime, Cefotaxime and Gentamicin, while 7 (50%) of the strains were sensitive to Chloramphenicol. However, 10 (71.4%) of the *Salmonella typhi* strains were determined to be resistant to Ampicillin and Co-trimoxazole, 9 (64.2%) resistant to Tetracycline and 6 (42.8%) resistant to Chloramphenicol (Table 1).

Table 1: Antibiotic susceptibility pattern of *Salmonella typhi* (n = 14)

Antibiotics	Sensitive	Intermediate	Resistant
Amikacin	14 (100.0%)	-	-
Ceftraxone	14 (100.0%)	-	-
Ciprofloxacin	14 (100.0%)	-	-
Enoxacin	14 (100.0%)	-	-
Ofloxacin	14 (100.0%)	-	-
Cefazolin/Cephradine	11 (78.5%)	-	3 (21.0%)
Ceftazidime	11 (78.5%)	-	3 (21.0%)
Cefotaxime	11 (78.5%)	1 (7.1%)	2 (14.2%)
Gentamicin	11 (78.5%)	-	3 (21.0%)
Chloramphenicol	7 (50.0%)	1 (7.1%)	6 (42.8%)
Tetracycline	5 (35.7%)	-	9 (64.2%)
Co-trimoxazole	3 (21.0%)	1 (7.1%)	10 (71.4%)
Ampicillin	3 (21.0%)	1 (7.1%)	10 (71.4%)

Table 2: Multi-dmg resistance pattern of *Salmonella typhi A* (n = 14)

Combination of Antibiotics	Resistant strains
Ampicillin+Co-trimoxazole	10 (71.4%)
Ampicillin+Tetracycline	9 (64.2%)
Ampicillin+Chloramphenicol	9 (64.2%)
Co-trimoxazole+Tetracycline	6 (42.8%)

Table 3: Antibiotic susceptibility pattern of *Salmonella paratyphi A* (n = 8)

Antibiotics	Sensitive	Intermediate	Resistant
Amikacin	8 (100.0%)	-	-
Ceftraxone	8 (100.0%)	-	-
Ofloxacin	7 (87.5%)	-	1 (12.5%)
Ceftazidime	7 (87.5%)	-	1 (12.5%)
Cefotaxime	6 (75.0%)	1 (12.5%)	1 (12.5%)
Enoxacin	6 (75.0%)	-	2 (25.0%)
Gentamicin	6 (75.0%)	-	2 (25.0%)
Chloramphenicol	5 (62.5%)	1 (12.5%)	2 (25.0%)
Ciprofloxacin	5 (62.5%)	-	3 (37.5%)
Tetracycline	4 (50.0%)	-	4 (50.0%)
Cefazolin/Cephradine	4 (50.0%)	-	4 (50.0%)
Ampicillin	4 (50.0%)	-	4 (50.0%)
Co-trimoxazole	3 (37.5%)	-	5 (62.5%)

Table 4: Multi-dmg resistance pattern of *Salmonella paratyphi A* (n = 8)

Combination of Antibiotics	Resistant strains
Co-trimoxazole+Ampicillin	4 (50%)
Co-trimoxazole+Tetracycline	4 (50%)
Co-trimoxazole+Cefazolin/Cephradine	4 (50%)

As for the multi-drug resistance pattern, these *Salmonella typhi* isolates also showed resistivity following exposure to a combination of drugs as well. Ten (71.4%) of the strains were resistant to a combination of Ampicillin plus Co-trimoxazole, 9 (64.2%) resistant to Ampicillin plus Tetracycline and Ampicillin plus Chloramphenicol and 6 (42.8%) of the strains were resistant to Co-trimoxazole plus Tetracycline combination (Table 2).

Regarding the 8 isolates of *Salmonella paratyphi A* identified in this study, all were found sensitive to Amikacin and Ceftraxone. Seven (87.5%) of the strains were sensitive to Ofloxacin and Ceftazidime, while 6 (75%) were determined to be sensitive to Cefotaxime, Enoxacin and Gentamicin. On the other hand, 5 (62.5%) of the isolates were found to be resistant to Co-trimoxazole and 4 (50%) resistant to Ampicillin, Cefazolin/Cephradine and Tetracycline (Table 3).

Antibiotic combination therapy also revealed resistant strains as 4 (50%) of the *Salmonella paratyphi A* isolates showed resistivity to a combination of Co-trimoxazole plus Ampicillin, Co-trimoxazole plus Tetracycline and Co-trimoxazole and Cefazolin (Table 4).

DISCUSSION

The antibiotic era has gradually failed to conquer the *Salmonella* infections in man. Since the demonstration of its efficacy in 1948, Chloramphenicol has been the drug of choice for salmonellosis. Although simultaneous plasmid specified resistance to Chloramphenicol, Ampicillin and sulphonamides was reported in an outbreak of typhoid fever in Central Mexico in 1972 involving more than 10,000 cases with many fatalities, it was not until early 1987 that the first resistant strains of *Salmonella typhi* was reported in Pakistan[9].

This study under discussion also revealed the prevalence of resistant strains of *Salmonella typhi* to Ampicillin (71.4%), Co-trimoxazole (71.4%), Tetracycline (64.2%) and Chloramphenicol (42.8%). These findings are consistent with those reported by other researchers working in the developing countries such as India and Bangladesh[12,13] as well as in the developed nations like United Kingdom[6] and Greece[14], which receive a regular flow of migrating workers particularly from India, Bangladesh and Pakistan. Researchers working on salmonellosis in Pakistan have also reported similar resistance patterns of *Salmonella typhi* to Chloramphenicol throughout the country[15,16].

However, researchers in other developing countries like Sri Lanka[17] have reported lower resistance levels of *Salmonella typhi* to Chloramphenicol. Similarly, workers

in developed countries like the Unites States[18] and Italy[10] have documented reduced levels of resistance to Chloramphenicol. This contrast to the findings of the present study may be due to the controlled use of this antibiotic in developed countries and the stringently enforced requirement of conducting a drug susceptibility test prior to the prescription of antibiotics. Such practices are circumvented in many developing countries with large, uneducated populace, where the infrastructure does not support the demand of adequate healthcare[5,9].

This study also revealed a high incidence of Ampicillin and Co-trimoxazole resistant strains of *Salmonella typhi*. Such findings are in confirmation with the reported documented by various studies carried out in Pakistan and in other South Asian countries[6,19,20]. These findings, despite being higher than those reported previously by many researchers[18,21], are however lower than those documented by other researchers[22,23]. Such contrasting results are indicative of the gradual development of resistant strains among pockets of communities throughout this region, as more and more people are forced into economic slavery and are transformed into economic refugees, or displaced because of civil strife, famine or natural calamities, migrating from rural to urban centers within the same country or crossing international borders hoping for a better life. Such happenings, which are quite regular in developing nations, have also resulted in the development of multi-drug resistant strains of pathogens like *Salmonella typhi*[24-26].

Determinants of antibiotics (Ampicillin and Co-trimoxazole) resistance may disseminate as a result of the cloned spread of epidemic strains, the spread of epidemic plasmids, or as a result of resistance genes migrating to many different strains and plasmids by transpositions[27]. The role of plasmids and transposons in multi-resistant *Salmonella typhi* isolated at Rawalpindi/ Islamabad has been well documented[28].

The current study further augments the argument of the emergence of multi-drug resistant strains of *Salmonella typhi* as it revealed a marked presence of *Salmonella typhi* strains resistant to the combined therapy of Ampicillin plus Co-trimoxazole; Ampicillin plus Chloramphenicol and Ampicillin plus Tetracycline. Other researchers, who have documented similar results, support these findings[7,12,22].

The study under discussion has also revealed the development of resistant strains to Tetracycline alone as well. The findings are quite high, 64.2% of the isolates were found resistant to Tetracycline, as compared to those reported in developed countries[10,18] and is once again reflective of the common practices and cultural acceptability of self-medication and purchasing of prescription drugs from over the counter, without proof of prescription in Pakistan[5].

This study revealed that quinolones might become the leading therapeutic agents against typhoid, as all *Salmonella typhi* isolates found sensitive to Ofloxacin, Ciprofloxacin and Enoxacin. Similar findings have also been reported by other researchers[13,16,22] who also in turn argue that fluoroquinolones have now become the first choice drug for treatment of multi-drug resistant typhoid fever due to their efficacy, oral route of administration and low cost compared to the third generation cephalosporins. But in developing countries like Pakistan, where drugs are easily available and purchased over the counter, it is inevitable that a day will arrive when researchers report on the emergence of multi-resistant *Salmonella typhi* strains that have developed resistance to quinolones as well[6,9,24].

Till such a day, which may not be far off into the future, it is safe to assume, based on the findings of this study, as well as other reported documents, that apart from quinolones, third generation cephalosporins, like Ceftraxone and aminoglycosides like Amikacin, may be used as therapeutic agents against the typhoid bacterium. The results of this study revealed that all the isolates of *Salmonella typhi* were sensitive to these drugs, which corresponds to the results documented by other researchers working in Pakistan and elsewhere[10,13,18].

The process of unrestricted usage of antimicrobes in developing countries is also a major contributory factor in the development of drug resistance in *Salmonella paratyphi A* as well and poses a therapeutic problem in the form of emerging multi drug resistant *Salmonella typhi* and *paratyphi* strains[27]. The first line therapeutic regimens of Chloramphenicol, Ampicillin and Co-trimoxazole have been rendered more or less ineffective in Pakistan in a significant number of enteric fever cases[16]. Studies in Pakistan and in the South Asian region have reported *Salmonella paratyphi* isolates resistant to conventional antibiotics used in typhoid cases[5,25].

In this study as well, 62.5% of the *Salmonella paratyphi A* isolates were resistant to Co-trimoxazole and 50% were resistant to Ampicillin and Tetracycline, while 25% resistant to Chloramphenicol. These findings conform to the reports documented in recent study[8]. The apparent increase of drug resistance among *Salmonella paratyphi* A, may have developed over the past six years.

To exacerbate matters, the strains of *Salmonella paratyphi A* isolated in this study were reported to have resistance to the antibiotic combination therapy as well. Of these multi-drug resistant isolates 50% showed

resistivity to the combined therapy of Co-trimoxazole plus Ampicillin, Co-trimoxazole plus Tetracycline and Co-tromixazole plus Cefazolin.

The emerging resistant of *Salmonella paratyphi A* to fluoroquinolones (Ofloxacin, Ciprofloxacin and Enoxacin) also reported in this study conforms to the documented findings of other researchers from patients residing in Rawalpindi[9], but is in sharp contrast to the reports submitted by other researchers where isolates were found to be sensitive to these fluoroquinolones[29]. However, these *Salmonella paratyphi A* strains were isolated form patients not residing in Rawalpindi.

Cephalosporins seem to be most effective therapeutic agent against *Salmonella paratyphi A* as well, as all the isolated strains in this study were reported to be sensitive to Amikacin, which conforms to the findings of earlier studies[5].

All the isolates of *Salmonella paratyphi A* were also found to be sensitive to Ceftraxone, which is in agreement with findings reported by other researchers in Pakistan[8,22], as well as those reported from Bangladesh[30].

In Pakistan almost all antibiotics are available over counter and can be purchased without prescription. In addition, clinicians also often prescribe antibiotics without taking the necessary steps of conducting culture sensitivity tests to ensure the use of the most effective antibiotic. To combined problem, patients do not take the medicine in the prescribed dosage or for the required duration either because of the cost involved, or stop taking the medicine once they feel that they have recovered. Under such circumstance, the result of developing resistant strains is greatly enhanced[15,22,26].

The unlimited and irrational use of drugs will in all likelihood continue for quite some time. One sure way to combat such usage is to promote awareness, education and health programs among the youth in particular and the rural masses in general. Civil society groups, health departments and physician's organizations must coordinate their efforts to bring about a shift in cultural norms. Even in developed countries where self-medication by patient is kept in check, the emergence of multi drug resistance is rife, often attributed to the use of antibiotics to the feed of farm animals[26]. These practices are also common in Pakistan as well. Therefore, the agriculture and livestock departments must be made aware of the drawbacks associated with antibiotics being fed to animals.

The exact prevalence of multiple drug resistant *Salmonella typhi* and *Salmonella paratyphi A* in Pakistan is not known, as there is a lack of proper microbiology laboratories at the district level in the country. Moreover, the rapid spread of the transferable resistance plasmid from one *Salmonella typhi* and *paratyphi* to the next is a very frightening scenario, which may increase the burden of this serious problem on the already under-staff, inadequately equipped healthcare infrastructure. We are aware that Chloramphenicol, Ampicillin and Co-trimoxazole are misused for trivial infections and prescribed to patients regardless of whether the prescription of drugs is validated, or not. The use of these antibiotics in chicken feed is going to bring havoc of resistant *Salmonella typhi* and *Salmonella paratyphi* A, if left uncontrolled and unmonitored.

Typhoid vaccinations on a mass scale is not feasible and provision of safe drinking water and improved sanitary systems are also unfortunately not going to be available in the immediate future, despite all the efforts of the government. The question that remains to be answered, is that how, under the current inflation rates, will people afford treatment of salmonellosis using the third generation cephalosporins when widespread resistance to flouoroquinolones becomes a common problem?

The answer is quite complex. However, what is quite simple is that because of the alarming increase in multi-drug resistant *Salmonella typhi* and *paratyphi A* to conventional antibiotics, typhoid fever must be treated very cautiously and newer antibiotics must only be used when they are indicated so as to prevent the further development of microbial resistance against them.

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