

# Changing trends in drug resistance among typhoid salmonellae in Rawalpindi, Pakistan

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الاتجاهات المتغيرة لمقاومة السلّمونيلات التيفية للأدوية، في روالپنڈي، باكستان

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**الخلاصة:** قام الباحثون بتحليل سجلات مزارع الدم التي أجريت في معهد الباثولوجيا التابع للقوات المسلحة في روالپنڈي، في ما بين عامي 1996 و2003، واستفردوا 477 من السلّمونيلات التيفية *Salmonella typhi*، و410 من السلّمونيلات نظيرة التيفية *S. paratyphi* A، من النمط «أ»، وذلك من عينات دم المرضى بالحمى التيفية. ولاحظنا وجود انزياح كبير في توزع السلّمونيلات التيفية وحساسيتها لمضادات الميكروبات. وقد انخفض معدل الاستفراء للسلّمونيلات التيفية بشكل كبير، بينما كانت السلّمونيلات نظيرة التيفية من النمط «أ»، تنبثق كممرضات *pathogen* رئيسية. وتقلصت المقاومة للأدوية التقليدية المضادة للتيفية في السلّمونيلات التيفية بشكل هائل من 80% إلى 14%، بينما ارتفعت مقاومة السلّمونيلات نظيرة التيفية، النمط «أ»، من 14% إلى 44%، وانخفضت الحساسية لعقارات الفلوروكينولون لدى النوعين. ولم يتم اكتشاف أي مقاومة للسيفالوسبورين من الجيل الثالث. ويبدو أن الاستخدام الواسع بلا قيد ولا شرط للأدوية الكينولونية هو السبب الأساسي وراء حدوث هذه الاتجاهات.

**ABSTRACT** We analysed the record of blood cultures carried out at the Armed Forces Institute of Pathology, Rawalpindi between 1996 and 2003. We isolated 477 *Salmonella typhi* and 410 *S. paratyphi* A from blood of patients suffering from typhoid fever. We observed a significant shift in the distribution and antimicrobial susceptibility of typhoid salmonellae. The isolation rate of *S. typhi* fell significantly while *S. paratyphi* A is emerging as a major pathogen. Resistance to conventional antityphoid drugs in *S. typhi* decreased dramatically from 80% to 14%, while in *S. paratyphi* A resistance increased from 14% to 44%. Susceptibility to the fluoroquinolones decreased in both. No resistance to third generation cephalosporins was detected.

## Évolution de la résistance aux médicaments chez les salmonelles typhiques à Rawalpindi (Pakistan)

**RÉSUMÉ** Nous avons analysé le dossier des hémocultures réalisées à l'Institut de Pathologie des Forces armées de Rawalpindi entre 1996 et 2003 : 477 *Salmonella typhi* et 410 *S. paratyphi* A ont été isolées dans le sang de patients atteints de fièvre typhoïde. Nous avons observé un changement significatif dans la distribution et la sensibilité aux antimicrobiens des salmonelles typhiques. Le taux d'isolement de *S. typhi* a significativement diminué tandis que *S. paratyphi* A devient un agent pathogène majeur. La résistance de *S. typhi* aux médicaments classiques contre la fièvre typhoïde a diminué considérablement, passant de 80 % à 14 %, tandis que la résistance de *S. paratyphi* A a augmenté, passant de 14 % à 44 %. La sensibilité aux fluoroquinolones a diminué pour les deux. Aucune résistance aux céphalosporines de troisième génération n'a été décelée.

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## Introduction

Typhoid salmonellae are important human pathogens. They thrive in overcrowded and unsanitary conditions. While improving sanitation and introduction of antibiotics has led to control of typhoid fever in the developed countries, it continues to plague the developing world, especially South Asia. The problem has been compounded by the development of plasmid-mediated antibiotic resistance against conventional antityphoid drugs, which first emerged in the 1970s. Multidrug resistance, defined as simultaneous resistance to the 3 conventional antityphoid drugs (chloramphenicol, cotrimoxazole and ampicillin), rapidly spread all over the world, and by the mid 1990s it was widespread in *Salmonella typhi*. The situation was saved by the introduction of the fluoroquinolones, which are now the treatment of choice for typhoid fever [1]. Widespread and uncontrolled use of these drugs has, however, led to the emergence of resistance against them also [1–3].

Several recent studies from various parts of Pakistan have reported increasing susceptibility of *S. typhi* to the conventional antityphoid drugs [4,5]. Similar observations in our institute prompted us to review the distribution and antimicrobial drug susceptibility pattern of typhoid salmonellae isolated between 1996 and 2003 (T. Butt et al., unpublished data, 2003).

## Methods

The study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, which provides laboratory services to a 1500-bed tertiary care hospital in Rawalpindi and is the main reference laboratory in Northern Pakistan. Data from 1996 to 2003 were retrieved from our database and

analysed for changing trends using the chi-squared test.

Blood samples were collected from patients clinically suspected of having typhoid fever. The patients were of all ages and both sexes and had been referred from various civil and military hospitals in Rawalpindi and Islamabad. Five mL blood was inoculated into 45 mL brain heart infusion broth (Oxoid, Basingstoke, UK) and incubated aerobically at 37 °C for 7 days. Subculture on Columbia agar (Oxoid) containing 5% horse blood (blood agar) and MacConkey agar (Oxoid) was done on days 1, 2, 3 and 7. The isolates were identified by morphology and standard biochemical tests using API 20E galleries (bioMerieux SA, Lyon, France) and confirmed as *S. typhi* and *S. paratyphi* A by serological tests using standard antisera (Wellcome Reagents Ltd., Beckenham, UK).

Antimicrobial susceptibility testing was done on Mueller–Hinton agar (Oxoid) by the modified Kirby–Bauer disk diffusion technique according to the National Committee for Clinical Laboratory Standards criteria [6]. The antimicrobial disks used (all from Oxoid) were ampicillin (10 µg), chloramphenicol (30 µg), co-trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg) and ceftriaxone (30 µg). The sensitivity plates were incubated aerobically for 18–24 hours at 37 °C. *Escherichia coli* ATCC 25992 was used as the control strain. Minimum inhibitory concentration (MIC) of ciprofloxacin was determined by the Kirby–Bauer broth dilution technique in cases of therapeutic failure (failure of fever to settle despite 7 days of treatment with oral ciprofloxacin).

## Results

A total of 477 *S. typhi* and 410 *S. paratyphi* A isolates were detected between 1996 and

2003. Even though the number of blood cultures carried out for typhoid fever did not change significantly from during this period (3906 in 1996, 3737 in 1997, 3670 in 1998, 3048 in 1999, 3583 in 2000, 3866 in 2001, 3872 in 2002, and 3422 in 2003), the isolation rate of typhoid salmonellae decreased significantly from 147 in 1996 to 49 in 2003 ( $P < 0.0001$ ). However, the proportion of *S. paratyphi* A isolates increased compared to *S. typhi* ( $P = 0.033$ ) (Figure 1).

A significant decrease in multidrug resistance was noted among the isolates of *S. typhi* which decreased from 80% in 1996 to 13% in 2003 ( $P < 0.0001$ ), while at the same time multidrug resistance among *S. paratyphi* A increased from 14% in 1996 to 44% in 2003 ( $P = 0.004$ ) (Figure 2). The resistance pattern of the isolates is presented in Tables 1 and 2.

Ciprofloxacin MICs were determined in isolates from 25 cases of therapeutic failure with oral ciprofloxacin between 2001 and 2003. Reduced susceptibility to ciprofloxacin (MIC 0.125 µg/mL–1.0 µg/mL) [3] was noted in 22 typhoid salmonellae, while 3 cases responded to parenteral ciprofloxacin.

No resistance was detected against ceftriaxone (Tables 1 and 2).

## Discussion

Several interesting trends were observed in our study. We noticed a decrease in the isolation rates of both *S. typhi* as well as *S. paratyphi* A over the past 8 years. There is, however, no evidence to suggest that the incidence of typhoid fever has gone down, as the various factors responsible for the endemicity of typhoid in Pakistan like poverty, overcrowding and absence of clean drinking water have not changed.

In Pakistan, antibiotics are easily available over the counter. This has led to widespread and uncontrolled use of quinolones as empiric treatment and self-medication for typhoid fever on the basis of clinical suspicion. The unrestricted use of quinolones is probably responsible for the falling isolation rate of typhoid salmonellae in the laboratory.

Another trend has been the increase in the relative proportion of *S. paratyphi* A isolates compared to *S. typhi*. Hannan et

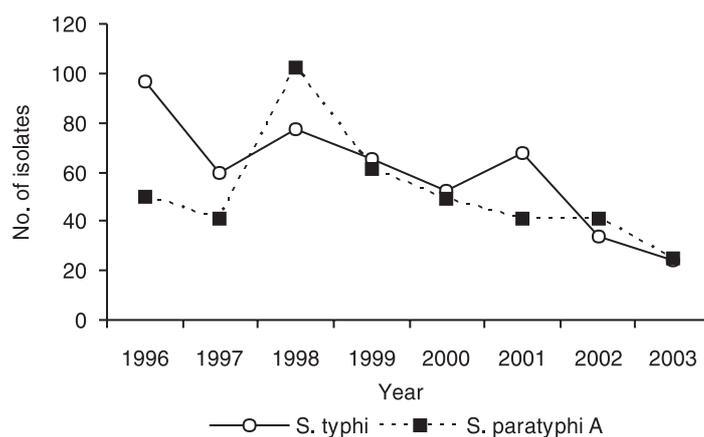


Figure 1 Isolation rates of typhoid salmonellae in Rawalpindi (1996–2003)

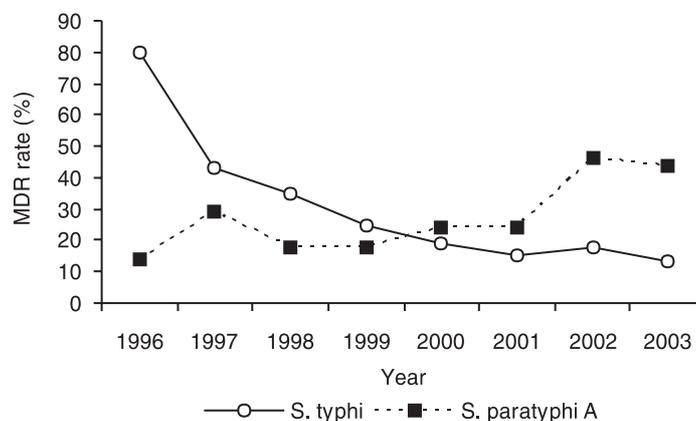


Figure 2 Trends of multidrug resistance (MDR) among typhoid salmonellae in Rawalpindi (1996–2003)

al. were among the first to test in vitro and in vivo efficacy of fluoroquinolones against typhoid salmonellae in 1986–87 and reported higher MICs of fluoroquinolones against *S. paratyphi A* even though they were within the susceptible range [7,8]. It

is possible that the indiscriminate use of quinolones, which has led to the suppression of *S. typhi*, has allowed the relatively resistant *S. paratyphi A* to occupy the niche vacated by *S. typhi*. It is also possible that increasing awareness and higher index of

Table 1 Antimicrobial drug resistance among *Salmonella typhi* (1996–2003)

Year	No of isolates resistant (%) to various antimicrobials					
	Amp	Cot	Cap	MDR <sup>a</sup>	Cip <sup>b</sup>	Ctx <sup>c</sup>
1996 (n = 97)	81 (83)	80 (82)	81 (83)	78 (80)	–	Nil
1997 (n = 60)	32 (53)	28 (47)	27 (45)	26 (43)	–	Nil
1998 (n = 77)	28 (36)	29 (38)	29 (38)	27 (35)	–	Nil
1999 (n = 65)	31 (48)	18 (28)	34 (52)	16 (25)	–	Nil
2000 (n = 52)	14 (27)	16 (31)	18 (35)	10 (19)	–	Nil
2001 (n = 68)	10 (15)	10 (15)	12 (18)	10 (15)	6 (9)	Nil
2002 (n = 34)	6 (18)	6 (18)	7 (20)	6 (18)	5 (15)	Nil
2003 (n = 24)	5 (21)	4 (18)	5 (21)	3 (13)	3 (13)	Nil

MDR = multidrug resistance.

<sup>a</sup>Simultaneous resistance to ampicillin (Amp), co-trimoxazole (Cot) and chloramphenicol (Cap).

<sup>b</sup>Reduced susceptibility to ciprofloxacin (minimum inhibitory concentration 0.125 µg/mL–1.0 µg/mL).

<sup>c</sup>Ceftriaxone.

Table 2 Antimicrobial drug resistance in *Salmonella paratyphi* A (1996–2003)

Year	No of isolates resistant (%) to various antimicrobials					
	Amp	Cot	Cap	MDR <sup>a</sup>	Cip <sup>b</sup>	Ctx <sup>c</sup>
1996 (n = 50)	8 (16)	7 (14)	7 (14)	7 (14)	–	Nil
1997 (n = 41)	22 (54)	15 (37)	14 (34)	12 (29)	–	Nil
1998 (n = 102)	32 (34)	24 (24)	20 (20)	18 (18)	–	Nil
1999 (n = 61)	27 (44)	13 (21)	27 (44)	11 (18)	–	Nil
2000 (n = 49)	16 (33)	12 (24)	12 (24)	12 (24)	–	Nil
2001 (n = 41)	14 (34)	11 (27)	13 (32)	10 (24)	2 (5)	Nil
2002 (n = 41)	20 (49)	19 (46)	23 (56)	19 (46)	3 (7)	Nil
2003 (n = 25)	14 (56%)	11 (44)	14 (56)	11 (44)	3 (11)	Nil

MDR = multidrug resistance.

<sup>a</sup>Simultaneous resistance to ampicillin (Amp), co-trimoxazole (Cot) and chloramphenicol (Cap).

<sup>b</sup>Reduced susceptibility to ciprofloxacin (minimum inhibitory concentration 0.125 µg/mL–1.0 µg/mL).

<sup>c</sup>Ceftriaxone.

clinical suspicion is responsible for more thorough investigation and increased diagnosis of paratyphoid fever. Changing host susceptibility and enhanced virulence of the pathogen could also be responsible for emergence of this pathogen. The same trend has also been observed in India [9,10].

The first case of multidrug resistance *S. typhi* in Pakistan was reported in 1987 [8]. Over the next 8 years, multidrug resistance in *S. typhi* isolated at the Armed Forces Institute of Pathology, Rawalpindi, rapidly increased to 75% [11]. Our study has shown several changing trends in the antimicrobial susceptibility of typhoid salmonellae in Rawalpindi. There has been a dramatic reduction in multidrug resistance rates among *S. typhi* isolates (Figure 2). Similar trends have been noted in other parts of the country [4,5] and elsewhere in the world [12,13]. The rate at which bacteria acquire resistance to antimicrobials is directly related to their exposure to these drugs [14,15]. Increasing resistance to the conventional antityphoid drugs has led to their replacement with fluoroquinolones and removal of the selective drug pressure

on the salmonellae and gradual reversal of the susceptibility pattern.

However, the same was not observed in *S. paratyphi* A—44% of our *S. paratyphi* A isolates were multidrug resistant in 2003. Resistance to the conventional antityphoid drugs has seen a steady rise since 1995 when the first case of multidrug-resistant *S. paratyphi* A in Pakistan was isolated at our institute [16]. The increasing resistance to conventional antityphoid drugs in the absence of selective drug pressure appears paradoxical. It is possible that as the pathogen has emerged, it is the already resistant clones that have spread. Conversely, the increasing resistance might be due to acquisition of resistance plasmids from other Gram-negative rods [14].

It seems that *S. paratyphi* A is now emerging as a major pathogen in Pakistan. Although considered to be a pathogen of low virulence, its diagnosis is also easily missed due to milder and more varied clinical presentation (compared to *S. typhi*). As mentioned earlier, MICs of fluoroquinolones against *S. paratyphi* A have been on the higher side ever since the introduction

of these drugs into clinical practice. This, when combined with selective drug pressure of unrestricted quinolone use, might lead to the rapid spread of quinolone-resistant *S. paratyphi* A.

Development of quinolone resistance in typhoid salmonellae is most worrisome [3,17–19]. We reported the first case of fluoroquinolone treatment failure in typhoid salmonellae in Pakistan in 1993 [20]. Since then the incidence of such cases has been increasing: 22 of our isolates between 2001 and 2003 exhibited reduced susceptibility to ciprofloxacin. The problem is compounded by the inability to identify this reduced susceptibility by the standard disk diffusion techniques. The inadequacy of the current guidelines for detection of this resistance has prompted a reappraisal and revision of these guidelines. We have recommended revised break-point ciprofloxacin MICs of 0.125 µg/mL–1.0 µg/mL as reduced susceptibility [3]. It is interesting to note that the first case of multidrug resistance *S. paratyphi* A in Pakistan, isolated in 1995,

had ciprofloxacin MIC of 0.125 µg/mL [16] which would make the isolate resistant to ciprofloxacin according to the revised guidelines.

To summarize, there is a definite shift in the distribution as well as antimicrobial susceptibility of typhoid salmonellae in Pakistan. *S. paratyphi* A resistant to both the conventional antityphoid drugs as well as the fluoroquinolones is emerging as a major pathogen. While resistance to the conventional antityphoid drugs in *S. typhi* has decreased dramatically, it is increasing against the fluoroquinolones. The common denominator in all these findings appears to be widespread and uncontrolled use of fluoroquinolones. Fluoroquinolones are the most effective antityphoid drugs available. It is imperative that these drugs are used judiciously to prevent the spread of this resistance. At the same time, the role of conventional antityphoid drugs in the treatment of *S. typhi* infection should be re-evaluated.

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