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Prevalence of *Salmonella typhi* and *Paratyphi* in urine cultures and their antibiotic sensitivity patterns in karachi

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Abstract

Background: To ascertain the prevalence of *Salmonella typhi* and *S. paratyphi* serotypes in urine, their role in causing urinary tract infection and their antibiotic sensitivity patterns.

Methods: A retrospective study was conducted on isolates grown on CLED, Chocolate agar and EMB medium from routine patient urine specimens over a period of six months from Jan-July 2012 in a private clinical lab in Karachi. Pertinent antibiotic susceptibility profiles of the Salmonella isolates were established using Kirby Bauer disc diffusion method on Mueller Hinton agar.

Results: A total of 41 specimens yielded three serotypes of enteric fever *Salmonellae* among 2252 samples of urine (1.82%) cultured during the study period from Jan-July 2012. This finding was more commonly seen in female samples, especially those of elderly women. Approximately 80.4% of these individuals had significant bacteriuria with viable counts of 104/ml urine or greater. Cefixime, cefotaxime and ceftriaxone exerted satisfactory antibiotic effect while significant resistance was seen to the fluoroquinolones ciprofloxacin (24.4%) and ofloxacin (21.6%), and cotrimoxazole (38.5%).

Conclusions: Salmonellae in urine and their possible role in causing urinary tract infection were assessed. The preferred empirical choice of drugs for treating salmonella UTI was ascertained.

Keywords: *Salmonella typhi* and *S. paratyphi*; Bacteriuria; Urinary Tract Infection; Antibiotic susceptibility

1. Introduction

Typhoid fever affects about 22 million people worldwide every year with an estimate of at least 200,000 deaths annually.¹ Its prevalence is especially high in developing countries; in Pakistan, the incidence has been reported to be about 412 cases per 100,000 individuals each year, making it apparently the fourth most common source of mortality in the nation when not adequately treated.²

The foremost causative agent of enteric fever is *Salmonella typhi* although serotypes of *S. paratyphi* A and *S. paratyphi* B are also involved.³

The transmission of enteric fever bacilli occurs via the feco-oral route from where they gain entry into the lymphatics and then the blood stream.³ Entry of Salmonella into the urine is a comparatively rare event,⁴ but when it occurs it may cause urinary tract infection (UTI).⁵

Salmonella can enter the urinary tract either by direct invasion of the bladder via the urethra or hematogenously.⁵ Also, *S. typhi* can be found in urine from patients who are chronic carriers of the organism with the involvement of urinary system, or from patients who had recently suffered from typhoid.⁵ Interestingly, some patients might show no previous history of typhoid fever.⁶

The main purpose of our study is to ascertain the prevalence of typhoidal serotypes in urine cultures of a cross-section of subjects in Karachi referred with suspected UTI to a local clinical diagnostic laboratory, and the inference focused by antibiotic resistance patterns of these isolates as compared with those of reported blood culture isolates. Also, the dearth in literature of studies done in Pakistan concerning this aspect of salmonella contagion in urine encourages our exercise.

2. Materials and methods

A retrospective study was conducted screening urinary isolates grown over a period of six months from Jan-July 2012 in a local clinical diagnostic laboratory and its branches in key areas of Karachi Metropolis. The urine specimens of patients presenting with suspected UTI referred by nearby physicians, clinics and hospitals were inoculated with calibrated wire loop

on Cysteine lactose Electrolyte deficient agar (CLED), Chocolate agar and EMB medium (Oxoid, UK) and incubated for 18-24 hours at 37°C . Among the 2252 isolates, a total of 41 identified as species of Salmonella on the basis of conventional morphological characteristics and biochemical reactions using API-20 E cupules and confirmed with specific Salmonella antisera (Difco, USA), were selected.

Antibiotic susceptibility of these isolates was done by Kirby-Bauer disc diffusion method on Mueller Hinton agar (Oxoid, UK) using standard ampicillin, cefixime, cefotaxime, ceftriaxone, co-trimoxazole, ciprofloxacin and ofloxacin commercial antibiotic discs.

Pyuria (WBCs/ml) was routinely estimated by microscopy of centrifuged deposit from 10ml of each patient urine specimens

3. Results & Discussion

Among the 41 salmonella isolates in urine specimens, *S. paratyphi*A was more often identified (36.6%) followed by *S. typhi*(34.1%) and *S. paratyphi B* (29.3%) noted in Table 1.

Table 1: Salmonella isolates (n=41) in urine specimens

| Serotypes | Presence in urine (%) |
|-----------------------|-----------------------|
| <i>S. paratyphi</i> A | 36.6 |
| <i>S. paratyphi</i> B | 29.3 |
| <i>S. typhi</i> | 34.1 |

Table 2 portrays the relationship of age and gender of the individuals whose urines yielded salmonella serotypes. Females (78%) significantly outnumbered the male patients (22%) and the incidence of serotype positivity appeared proportional to increasing age in both genders. Approximately 17.07% of the children were less than 10 years of age; among them, 14.63% were female and only 2.4% were male. Even among those who were above 40 years of age, as many as 41.46% of the individuals were female, only 9.76% male.

Table 2: Relationship of age and gender with isolates

| Age (years) | Male % | Female % | Total % |
|-------------|--------|----------|---------|
| < 10 | 2.44 | 14.63 | 17.07 |
| 10-40 | 9.76 | 24.39 | 34.15 |
| > 40 | 9.76 | 41.46 | 51.22 |
| Total | 22 | 78 | 100 |

The viable bacterial count (VBC) associated with amount of pyuria/hpf is listed in Table 3. A total of 4.88% and 14.63% of the patients had scanty VBC's of 10²/ml and 10³/ml of urine respectively, and none of them had suggestive pyuria>10/ml. However, approximately 46.34% of individuals with moderate (VBC 10⁴/ml) and significant bacteriuria (10⁵/ml or more) had substantial pyuria amounts >10 WBC's/ml in their urine specimens.

Table 3: Bacteriuria and pyuria associated with Salmonella isolates in urine

| | 0 | 4.88 | 4.88 |
|-------|-------|-------|-------|
| 102 | 0 | 14.63 | 14.63 |
| 103 | 7.31 | 9.76 | 17.07 |
| 104 | 29.27 | 19.51 | 48.78 |
| 105 | 9.76 | 4.88 | 14.63 |
| >105 | 46.34 | 53.66 | 100 |
| Total | | | |

The sensitivity profiles of the isolates to eight relevant antibiotics (Table 4) indicated that the 3rd generation Cephalosporins (ceftriaxone, cefotaxime and cefixime) were

the most effective followed by co-amoxiclav, while significant resistance was observed to ampicillin (97.6%), cotrimoxazole (38.5%) and even the fluoroquinolones ciprofloxacin (24.4%) and ofloxacin (21.6%).

Table 4: Antibiotic sensitivity of Salmonella isolates

| Drugs | Sensitive % | Intermediate % | Resistant % |
|---------------|-------------|----------------|-------------|
| Ampicillin | 2.4 | 0 | 97.6 |
| Co-amoxiclav | 80.5 | 4.9 | 14.6 |
| Cefixime | 95.1 | 4.9 | 0 |
| Cefotaxime | 97.6 | 2.4 | 0 |
| Ceftriaxone | 97.6 | 2.4 | 0 |
| Ciprofloxacin | 73.2 | 2.4 | 24.4 |
| Ofloxacin | 73 | 5.4 | 21.6 |
| Cotrimoxazole | 53.8 | 7.7 | 38.5 |

The presence of Salmonella in urine is said to be a rare occurrence;⁴ however, our study showed a total of 41 out of 2252 individuals that tested positive for salmonella in their urine specimens. Three serotypes were identified. *S. paratyphi*A had the highest occurrence (36.6%) followed by *S. typhi* (34.1%) and *S. paratyphi B* (29.3%). This finding was inconsistent with two previous reports in which *S. typhi* was the most common serotype in infected urines, while *S. paratyphi* A and *S. paratyphi*B were less frequently isolated.^{5,7} However, both these studies were done elsewhere, and not in Pakistan.

Among the positive urine samples, a distinct gender partiality of salmonellae was noted, with 78% of individuals involved being female and only 22% were male. This gender penchant was consistent with previous reports in literature.^{8,9} In one of these studies conducted in the United States, there were 2.8 times more females with Salmonella bacteriuria as compared to males⁸, while our results showed females to be as much as 3.55 times more likely to develop salmonella bacteriuria. A short urethra in females is possibly one reason for this increased incidence in females⁵ and also the fact that females are more susceptible to develop carrier states of salmonella infection¹⁰ wherein the bacterium has a tendency to enter into the urine⁵.

Salmonella bacteriuria was noted in all age groups; however its incidence increased with increasing age. Approximately 17.07% individuals were under 10 years of age, while 34.15% individuals were between 10- 40 years of age and 51.22% were above 40 years of age. A likely reason for this increased incidence with age is possibly the development of chronic carrier state of salmonella infection which occurs particularly in the elderly.¹¹

Also, salmonella bacteriuria was more often seen in females above 40 (41.46%), a finding consistent with that observed in the United States which also showed pronounced incidence of salmonella bacteriuria in elderly females.⁸ The most probable reason for this increased incidence in elderly females is the fact that elderly females are more likely to become chronic carriers of salmonella.^{10,11}

Although the presence of salmonella in urine is a rare occurrence⁴, when it is present, it can activate UTI⁵. Bacteriuria provides valuable information in the diagnosis of a UTI¹² and significant bacteriuria is best assessed by urine culture with the formation of colony forming units (CFU).¹³ The greater the CFU the higher the positive predicted value (PPV) for UTI. A value of 10⁴/ml CFU or greater is suggestive of urinary tract infection¹⁴. Also, bacteriuria when combined with pyuria is even more diagnostic for UTI¹⁵. Pyuria, commonly defined as

the presence of 5-10 leukocytes/ml or more in centrifuged urine deposit suggests an ongoing inflammation of the urinary tract which most likely is due to bacteriuria¹³.

Although significant bacteriuria with pyuria is suggestive of UTI as implied in our exercise, a definite diagnosis of UTI, however, according to some researchers can be better made in the presence of clinical symptoms involving the urinary tract.^{13,14,16} Admittedly a limitation in our study is the lack of such data, since patients referred to a private clinical lab do not provide personal history, aside from name, sex and age; hence a diagnosis of UTI is implied with limitation. However, our observation that a majority of our patients did indeed have true descending salmonella UTI's, as opposed to fecal contamination, by virtue of the isolates being recovered in pure culture and in high concentrations, and many with significant coexisting pyuria..

Indeed, there were approximately 80.48% individuals that had VBC's of 104/ml urine or more in our study out of which 46.34% had suggestive and 34.14% had concomitant negative pyuria. Also, 63.41% had VBC's of 105/ml or greater; out of these, 39.02% individuals had suggestive pyuria and 24.39% had urines with negligible pyuria. Notably, 14.63% of patients had significant bacteriuria with VBC's greater than 105/ml urine; among these, 9.76% had pyuria while 4.88% had urinary pyuria in traces.

The antibiotic sensitivity patterns determined indicated that the 3rd generation cephalosporins ceftriaxone and cefotaxime were most effective (both 97.6%) against our salmonella isolates in urine, followed by cefixime (95.1%). While the isolates were least affected by ampicillin (97.6%) and cotrimoxazole (38.5%), an observation to be underlined was that 24.4% and 21.6% of the strains isolated were resistant to ciprofloxacin and ofloxacin respectively, the two most currently empirically prescribed drugs for treating enteric fever.^{13,17} This hence suggests an altered antibiotic sensitivity pattern of salmonella in urine compared with that of salmonella in blood; indeed this increased fluoroquinolone indifference of urinary isolates is consistent with that in other studies.^{17,18} The frequent fluoroquinolone administration in Typhoid fever and carrier states of Salmonella possibly accounts for fluoroquinolone-resistant strains emerging in urine.¹⁷

Ampicillin and co-amoxiclav showed contrasting antibiotic sensitivity patterns. Co-amoxiclav was indeed significantly more effective (80.5%), while ampicillin was least active, with 97.6% of the isolates resistant to this drug. High ampicillin resistance against salmonella is consistent with studies in India.^{19,20}

Finally, it may be mentioned that along with gram-negative bacteria, with *E. coli* in particular most commonly associated with UTI, the list of possible isolates encountered, at least in our environment where Enteric fever is endemic, should include Salmonella.

Conclusion

The presence of salmonella in urine, although a comparatively rare finding, is prevalent in our environment. The reason for this occurrence is unclear and needs to be elucidated.

S. typhi, *S. paratyphi* A and *S. paratyphi* B were identified in patient urines and their possible role in causing a UTI was assessed.

The isolates were more common in females, and especially those who were above 40 years of age who seem to be more prone to the infection.

Ampicillin in particular was least effective, while cefixime, cefotaxime and ceftriaxone showed satisfactory activity against the strains in urine. Notably, the commonly used drugs in treatment of UTI – the fluoroquinolones, showed notable inactivity and hence are not considered suitable drugs for empirical prescription for UTI caused by Salmonella.

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