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Case Study

Salmonella typhi: The Story beyond Typhoid

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Abstract

Background: Urinary Tract Infections (UTIs) caused by *Salmonella typhi* are rare, but there are a few reports of bacteria in urine from nontyphoidal *Salmonella*e and other Gram-negative bacilli. However, cases of bacteriuria with UTI due to nontyphoidal *Salmonella*e were more frequently reported in the past among individuals having urolithiasis. **Case Study**: We, herein, report a case of *Salmonella typhi* bacteriuria with urolithiasis in a woman aged 35 years who had a poor socio-economic background and came from the rural belt of western Nepal with inadequate and hygienically unsatisfactory drinking water facilities. **Results**: The woman's multiple episodes of vaginal bleeding led to an abdominal and pelvic ultrasound, which revealed several renal stones. The tests on both the stone and urine sample showed the presence of *Salmonella typhi*, and they reacted the same way to antibiotics. **Conclusion**: Our report highlighted the association of renal stones with urinary excretion of *Salmonella typhi*. Isolation of the organism from the crushed-out material of the renal stone as well as from the urine could suggest the persistence of the organism in the stone matrix being excreted from time to time, giving rise to intermittent bacteriuria. Based on the clinical, imaging, and lab results in this case, we, highlighted the importance of *Salmonella typhi* colonization on the stone with/without obvious clinical manifestations.

Keywords: *Biofilms; Salmonella; Urinary Stones; Typhoid Carrier*

Introduction

Typhoid is endemic in many parts of India and Nepal (Alfat *et al.*, 2020; Klosterman, 2014). *Salmonella Typhi*, though, happened to be the sole agent responsible for causing typhoid fever and sepsis; might cause extra-intestinal infections involving joints, soft tissue, spleen, and cardiopulmonary, hepatobiliary and genitourinary systems (Dawar *et al.*, 2017; Buchta & Dunn, 2003). Urinary tract infection (UTI) due to *Salmonellae*, a rare entity was shown to be caused by nontyphopidal *Salmonellae* such as *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella heidelberg*, and *Salmonella newport* (Mourani *et al.*, 2005). However, UTIs due to *Salmonella Typhi* were still rarer even in the areas endemic for typhoid fever (Mathai *et al.*, 1995).

At the same time, *S. typhi* bacteriuria in association with urolithiasis was documented earlier (Mourani *et al.*, 2005; Pandeya *et al.*,2006). This association could be addressed in two ways; UTI giving rise to urolithiasis or urolithiasis leading to subsequent episodes of UTI (Bichler *et al.*, 2002; Shafi *et al.*, 2013). Urolithiasis is a frequently encountered urological disorder highly prevalent in Nepal (Pandeya *et al.*, 2006). Reports from across the country emphasized not only its high prevalence rate, but also the

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frequent occurrence of the subsets of this condition like, nephrolithiasis, ureterolithiasis, and urinary bladder stones (Shah *et al.*, 2020).

Notwithstanding the above, studies on the pathogenesis of stone formation and mechanism of colonization of bacteria in the stone, and their antibiotic susceptibility patterns, still remain under-investigated. Over and above, urolithiasis due to *Salmonella typhi* was not reported from the country of Nepal ever in the past. Here, we, describe a case of *Salmonella typhi* bacteriuria associated with renal pelvic stone in a middle-aged woman who reported to the Manipal Teaching Hospital (MTH), Manipal College of Medical Sciences, a tertiary care centre in Western Nepal.

To the best of our knowledge, this is the first of its kind case report in Nepal on *Salmonella typhi* urinary carriage in association with renal stone.

Material and Methods

Case Report

A 35-year-old female presented to the Obstetrics and Gynecology Clinic of MTH in July 2022 with complaints of bleeding per vagina (PV) and lower abdominal pain since last one month. Initially, she had scanty vaginal bleeding, but later she experienced profuse episodes about a week before reporting to the hospital. She was afebrile and was hemodynamically stable. Her past history revealed that she had a high-grade fever a month prior to her present illness. This febrile state lasted for 5–6 days and was associated with chills, myalgia, anorexia, and headache. There was no history of any rash, nausea, vomiting, or cough. Her fever subsided after the intake of oral medications from the local pharmacy. She was taking medicine for her known hypothyroidism. One year prior to the present illness, she had undergone ultrasound-guided aspiration of a renal cyst. On further inquiry, she revealed that she lived in a rural area with poor sanitation and hygienically unsatisfactory and inadequate drinking water facilities, the source of drinking water for everyone in the locality being untreated and unboiled tap and river water.

The woman was checked for her bleeding PV with an ultrasound of her abdomen and pelvis, which unexpectedly revealed kidney stones on the right side along with swelling in a part of the kidney. She was then referred to the urology department for further investigation. The urologist recommended several tests, including kidney function tests, blood sugar estimation, a CT scan of the urinary system, and a standard urine routine microscopy.

Microbiological Investigations

The woman was also investigated for her febrile episode. Her urine, blood, and stool samples were subjected to culture and sensitivity, and the serum sample to the Widal test as per the standard protocol (Collee, Miles & Watt, 1996). Antibiotic sensitivity testing was performed using the Kirby-Bauer disc diffusion test, and the results were interpreted in accordance with the recommended guidelines (Bauer, 1966).

The peroperatively removed stone was transported to the laboratory with all sterile precautions using a sterile multipurpose container containing sterile normal saline. On reaching the laboratory, the contents of the multipurpose container along with the stone were decanted onto a sterile petri dish, and the stone was crushed with sterile forceps. The pulverized material was cultured onto blood agar and MacConkey agar plates and incubated at 37°C. Bacterial growth was identified, and antibiotic sensitivity patterns were determined in accordance with the standardized techniques mentioned earlier (Collee, Miles & Watt, 1996; Bauer, 1966).

Ethical Approval

For conducting all clinical radiological and laboratory investigations on the subject ethical approval was obtained from the institute review committee (IRC) of the Manipal College of Medical Sciences (MCOMS) vide its letter No. MCOMS/IRC/GA dared the 25th of July 2023. Written consent was taken from the patient for conducting the study.

Results

The woman's general physical examination was unremarkable without any evidence of hypertension, diabetes, or chronic pulmonary disease. The abdomen was soft and nontender. All vital functions were within normal limits. Renal function tests were normal, with serum urea 17 mg/dl (0.94 mmol/L) and serum creatinine 0.8 mg/dl (0.04 mmol/L). Her random blood sugar level was noted to be 95.6 mg/dl (5.31 mmol/L). CT IVU (computerized tomography intravenous urography) revealed a bulky uterus, a small right kidney with focal caliectasis within the lower pole, and right-sided nephrolithiasis with multiple calculi: 2 in the upper zone (11×8 mm and 2.2×1.5×2 mm), 2 in the middle zone (2×5 mm and 7×6.2 mm), and 1 in the lower zone (9.3×9.6 mm). A routine urine examination showed 10–12 WBCs/high power field, plenty of epithelial cells, and granular casts. Urine culture yielded growth of *Salmonella typhi* confirmed by standard biochemical tests and serotyping (Collee, Miles & Watt, 1996). The organism was sensitive to amikacin, cefotaxime, ceftazidime, ciprofloxacin, norfloxacin, cefoxitin, and cefoperazone sulbactam but resistant to nitrofurantoin. The stool culture did not show growth of any enteric pathogen, and the blood culture was sterile. The serum sample subjected to the Widal test, however, revealed the following antibody titers against the *Salmonella typhi* O and H antigens, respectively: TO 1:160 and TH > 1:320, quite suggestive of past infection due to *Salmonella typhi*. The patient was treated with ciprofloxacin 500 mg every 6 hours for a period of 7 days.

The DTPA scan to check kidney function (figure 1a, 1b) showed that the right kidney was working at 31% and the left kidney at 69%. Thereafter, right percutaneous nephrolithotomy (PCNL) with right DJ stent with polypectomy was planned after obtaining a sterile report of the repeat urine culture. The patient was operated on soon after the completion of 7 days' ciprofloxacin therapy and DTPA scanning for assessment of kidney functions. The stone removed during surgery (figure 2) was sent to the laboratory for bacteriological analysis along with one blood and one more urine sample for culture and sensitivity.

Figure 1a: Fluoroscopic guided PCNL

Figure 1b: Double J Stenting

Figure 2: Stone removed during surgery

After overnight incubation, blood agar showed medium-sized, opaque, moist, non-hemolytic colonies, and MacConkey agar showed moist, non-lactose-fermenting colonies (figure 3). On Gram staining, both the colonies happened to be Gram-negative bacilli, which were identified as *Salmonella Typhi* based on the biochemical test and serotyping results (Weissfeld *et al.,* 1998). Antibiotic susceptibility was performed (Bauer, 1966), and the sensitivity pattern of the organism was exactly the same as the one shown by the isolate from the preoperative urine sample. During the postoperative period, fresh blood and a urine sample were sent for culture, which did not yield any organism. The patient was discharged with the advice to continue ciprofloxacin for another seven days.

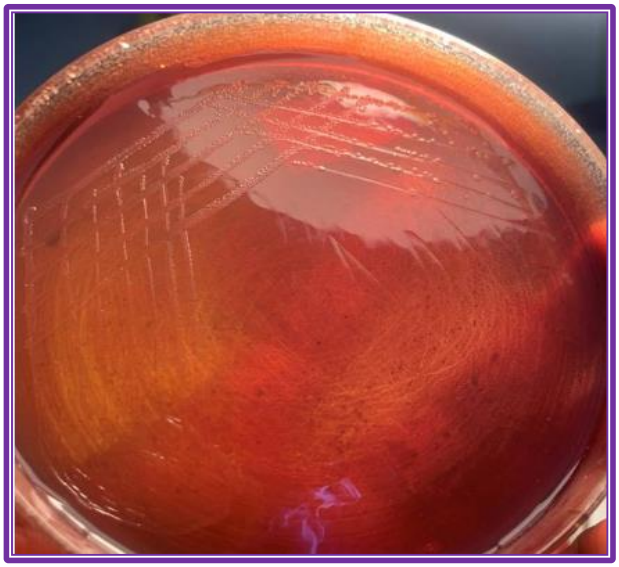

Figure 3: Mac Conkey agar showing non-lactose fermenting colonies (yielded both by the stone matrix culture and urine culture)

Discussion

Urinary tract infections due to *Salmonella* in general and *Salmonella typhi* in particular are unusual (Tavichakorntrakoo *et al.*, 2012). Nevertheless, *Salmonella* could enter the urinary tract either via the hematogenous route or by direct invasion of the urinary bladder through the urethra. In women, as in our case, shortness of the urethra is always considered the primary risk factor. Urinary tract infection (UTI) due to *Salmonella typhi*, though rare (Huang *et al.*, 2012), can occur when *Salmonella typhi* is isolated from the urine of individuals recovering from a recent episode of typhoid fever or from chronic urinary carriers following a past attack of typhoid fever (Mathai *et al.*, 1995).

The present case had a history of fever one month back, and she lived in a community with poor sanitation and an unsafe drinking water facility. The results of the Widal test performed a month after the febrile episode were quite suggestive of a typhoidal illness in the recent past. Therefore, all the aforementioned personal and past history, along with the serological evidence, suggested she might have suffered from typhoid fever and could possibly be a convalescent urinary carrier of *Salmonella typhi*. It was not a case of UTI per se due to *Salmonella*, as she had never presented with burning micturition or frank pain. Rather, bacteriuria was, perchance, discovered only during the routine investigations. Mathai *et al.*, (1995) reported a series of cases of asymptomatic bacteriuria in association with recent typhoid fever (Mathai *et al.*, 1995). *Salmonella typhi* bacteriuria was also reported among cases with structural and functional abnormalities of the urinary tract (Kapoor *et al.*, 1992; Maiwall *et al.*, 2020). The literature review suggested that such abnormalities could be a result of neoplasms of the kidney, hydronephrosis, anatomic abnormalities, tuberculosis, schistosomiasis, prostatic hypertrophy, renal transplant, and lupus nephritis. To add further to the list, a rare case of *Salmonella typhi* renal abscess was recently reported from India (Kaur *et al.*, 2015). However, many of

these cases did not have a previous history of typhoid fever, whereas our patient did have clinical, epidemiological, and laboratory evidence favoring a past episode of typhoid fever.

The present case had multiple renal stones. Nephrolithiasis predisposing to *Salmonella* bacteriuria was reported by others (Mathai *et al.*, 1995; Melzer *et al.*, 1965). However, the relationship between kidney stone disease and bacteriuria remained unclear, creating a paradox as to which was the trigger and which was the consequence. Lodgment of bacteria in the kidney might have the etiopathogenic role in the formation of infectious stones (magnesium ammonium phosphate or struvite stones frequently combined with calcium phosphate or calcium bicarbonate apatite) through the urea-splitting mechanism of urease-producing organisms such as *Proteus, Pseudomonas, Providencia, Ureaplasma*, and *Klebsiella* (Ripa *et al.*, 2022). However, recent reports, though scanty, on the possible role of some of the non-urease-producing enteric Gram-negative bacteria in the onset and development of renal stones are intriguing (Shah *et al.*, 2020; Ripa *et al.*, 2022). Whether it is urease-producing or non-urease-producing, it is always important to take into consideration that the same organism should be isolated both from the stone material and from the patient's urine. A comprehensive literature review showing the relationship between kidney stone disease and UTI has been depicted in Table 1.

Table 1: Association of kidney stone with UTI

Study population/papers reviewed	Key findings	Type of study (country)	Author, year
Meta -analysis by data base	UTI and KSD (kidney stone disease) were mutually co-existing. Surgical removal of stone was advocated when UTIs were recurrent	Systematic Review (Italy)	Ripa *et al.,* 2022
A 59 year old with a past history of nephrolithiasis and multiple episodes of UTIs due to non typhoidal *Salmonella*e (NTS)	UTIs due to NTS were usually associated with structural abnormalities. Nephrolithiasis and nephrocalcinosis were the major risk factors	Case Report (USA, Pakistan)	Altaf *et al.,* 2020
Two cases of chronic kidney disease with UTI due to *Salmonella Typhi*	Nephrolithiasis and nephrocalcinosis were the concomitant findings. In patients with structural and functional abnormalities of the urinary tract, *Salmonella* would be the probable cause of UTI.	Caser series (India)	Dawar *et al.,* 2017
88 cases of UTI	The association of microorganiems isolated from urine and nidus culture of urinary stones was significant and could predict the origin of infective stones. E coli, Proteus, and Klebsiella were the predominant organisms obtained from urine and stones	Perspective cohert study (Nepal)	Shah *et al.,* 2020
806 patients with urolithiasis comprising of two groups; UTI group and noninfectious group	Stone shape, size and multiple sites of stone formation could be considered important factors of UTI in patients with urolithiasis	Retrospective (China)	Yangzhi *et al.*, 2018
1679 patients <18 years with KSD and UTI	UTI was found to be commonest condition associated with KSD (34.1%)	Retrospective (Taiwan)	Huang *et al.*, 2012
100 patients with KSD and UTI	Prevalence of UTI associated with KSD was 36%	Prospective cohert study (Thailand)	Tavichakorntrakool *et al*, 2012
Salmonella bacteriuria with urolithiasis	Clinical significance of *Salmonella bacteriruria*	Case series (India)	Mathai *et al.*, 1995
Paediatric UTI with urolithiasis	Association of urolithiasis with UTI due to *Salmonella typhi*	Case report (Lebanon)	Mourani *et al.*, 2005
Nephrolithiasis in a 37year patient	Association of *Salmonella paratyphi* A with kidney stone	Case Report (KSA)	Al-Otaibi FE *et al.*, 2003;

Urinary stone culture and urine culture yielding the same organism was recently reported by Shah *et al.*, from a tertiary care hospital in Nepal, and the authors were of the view that stone formation was infection induced and that such conditions could arise due to alteration in the urinary enzymic activities leading to the formation of mineralizable stone matrix (Shah *et al.*, 2020). However, the presence of bacteria in general and *Salmonella typhi* in particular in the urinary passage leading to urolithiasis might be due to the organism per se causing UTI, an extremely rare phenomenon due to *Salmonella typhi* (Mourani *et al.*, 2005); or it could be as a result of the development of a urinary carrier state following an episode of typhoid fever. The latter could be a possibility in our case, based on the epidemiological, personal, and laboratory data stated above.

Our patient had urolithiasis, and *Salmonella typhi* was isolated both from the urine and the stone material. It was frequently debatable in the past whether lithiasis preceded the *Salmonella* carrier state, providing nidus for the organism to colonize on the stone surface, or whether it was secondary to urinary excretion after typhoid fever. The second proposition seemed unlikely in our case, as the patient had had an attack of febrile illness only a month or two prior to her present illness, and the stone size and texture were suggestive of the fact that the stone might have persisted for quite some time (Cotran *et al.*, 2003).

Melzer *et al.* (1965), reported a series of cases with clinically significant non-*Salmonella* UTI associated with nephrolithiasis. The authors believed that the existence of renal stones could predispose to the development of chronic *Salmonella* infection of the kidney. Others (Yongzhi *et al., 2018)* suggested that having bacteria in the urine of patients with kidney stones and a history of *Salmonella* infection would be a strong link. Such intimate relationships between renal calculi and microorganisms were mediated via the biofilm-forming ability of the bacteria on the stone surface (McLean, Stickler, & Nickel, 1996). Marcus and colleagues (Marcus *et al.*, 2008), in yet another study, reported that biofilms influenced the development of kidney stones, leading eventually to persistent and recalcitrant infection of the urinary tract with intermittent bacteriuria. It was also stated that mineralization and subsequent calculus formation were an aftereffect of biofilm-related infection (McLean, Stickler & Nickel, 1996; Marcus *et al.*, 2008).

However, these infected urinary stones often formed because urease-producing bacteria got stuck, which released ammonia, raised the urinary pH, and caused calcium and magnesium to settle as carbonate apatite and struvite.

These minerals could get trapped in the organic matrix that surrounded the infecting organisms, ultimately developing into the mature calculi. However, the mechanism adopted by the non-urease-producing bacteria to establish a niche for biofilm development on urinary stones is not exactly known, although various host and bacterial factors were proposed to have contributed to this (Stickler & Zimakoff, 1994).

As it may be, biofilms, in any clinical setting in general and in the case of renal stones in particular, could continually dislodge planktonic cells from the matrix-bound sessile community, causing intermittent shedding of bacteria in urine. This unique phenomenon of biofilm riddle would be well explained in our patient, who had urinary calculi and was excreting the bacilli in the urine. Contrarily, it was also interesting to note after literature review that individuals with gallstones were likely to become typhoid carriers because *Salmonella typhi* was able to form biofilms on the surface of gallstones (Prouty, Schwesinger & Gunn, 2002; González *et al.*, 2018; Di Domenico *et al.*, 2017).

The aforementioned concept of bacteria forming biofilms on stone matrix was further exemplified by other researchers (Tavichakorntrakoo *et al.*, 2012; Brain *et al.*, 2021) who believed that bacteria appeared to selectively attach to crystals and increased aggregation of crystals. Studies have demonstrated that the presence of bacteria could enhance the expression of stone matrix proteins, potentially initiating the formation of stones. It was therefore plausible that urinary stones might act as a foreign body, becoming colonised in the same way that a urinary catheter was invariably colonised. As only a small proportion of patients with colonised catheters would become symptomatic, it was

reasonable to assume that the same could apply to colonised urinary stones, with only a subset of patients developing symptomatic UTIs.

So, ours is the first report to document the clear evidence of *Salmonella typhi* excretion in urine and localization in kidney stone. It is, therefore, needless to emphasize that the present report should aid in the understanding of the *Salmonella* carrier state, which is an important but under-researched area of typhoid fever pathogenesis. Further studies are needed in order to understand the basis of such carrier state development, which may enable us to identify the effective strategies for the prevention and treatment of chronic carriage and infection.

Conclusion

Our report provided evidence that during convalescence from typhoid fever, *S. typhi* may colonize a pre-existing renal stone and could be intermittently excreted in the urine. Thus, routine urine culture should be advised in all cases of urolithiasis, lest there be any further complications.

Conflict of Interest

The authors declare that they have no competing interests.

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