SALMONELLA ARIZONAE: AN UNCOMMON UROPATHOGEN?

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ABSTRACT

Background: *Salmonella arizonae,* an uncommon uropathogen, is a potentially serious cause of urinary tract infection (UTI).

Objective: The aim of the study was to determine the prevalence of *Salmonella arizonae* among Gram-negative uropathogens in Sokoto, Nigeria.

Materials & Methods: A total of 365 urine samples were collected over a period of four months and examined to detect the presence of bacterial pathogens. Isolates were identified and their susceptibility to commonly used antibiotics was determined using the Microgen Identification Kit (GN-ID) and modified Kirby Bauer method, respectively.

Results: The results were interpreted according to the methods described by Clinical Laboratory Standard Institute (CLSI) in 2012. A total of 61 Gram-negative bacteria comprising 19 (31.15%) *E. coli*, 15 (24.59%) *Salmonella arizonae*, 7 (11.48%) *Klebsiella pneumoniae*, 3 (4.92%) *Klebsiella oxytoca*, 6 (9.84%) *Enterobacter gergoviae*, 4 (6.56%) *Citrobacter freundii,* 4 (6.56%) *Serratia marcescens,* and 1 (1.64%) each of *Enterobacter aerogenes, Proteus mirabilis* and *Edwardsiella tarda* were isolated. The results showed that 64.1% of the isolates were multidrug resistant (MDR). *Salmonella arizonae* showed a high degree of resistance to nalidixic acid, cotrimoxazole, gentamicin, norfloxacin, and amoxicillin/clavulanic acid. *Salmonella arizonae* were more sensitive to ciprofloxacin and nitrofurantoin.

Conclusion: Our findings showed a high prevalence of UTI with *Salmonella arizonae* isolated from urine in Sokoto Metropolis.

Keywords: Urinary tract infection, *Enterobacteriaceae*, *Salmonella arizonae*

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INTRODUCTION

Urinary tract infection (UTI) is the most common bacterial infectious disease in community practice, with a high rate of morbidity and financial cost[1]. It affects people of all ages, from neonates to the geriatric age group[2, 3]. UTI is even considered one of the most common bacterial diseases worldwide and is characterized by a wide range of symptoms, from mild irritative voiding to bacteremia, sepsis, or even death[4–10]. Bacteria are the major causative organisms and implicated in more than 95% of UTI cases[11]. About 80–85% of UTIs are caused by Gram-negative bacteria[12, 13].

Salmonella specie (spp) are Gram-negative bacilli and members of the *Enterobacteriaceae* family. *Salmonella enterica* sub-specie (ssp) *arizonae* is one of the less common sub-species of *Salmonella*[14]. *Salmonella arizonae* was first identified in Arizona in 1939; most cases have been reported in Southwestern US among a large Hispanic population that uses rattle snake products for medicinal purposes[15].

Salmonella arizonae was documented to be a pathogen that causes a spectrum of diseases in humans and animals, including domesticated and wild animals, reptiles, birds, and insects[16]. The organism is part of the normal reptile intestinal flora but can cause disease in

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monotremes, turkeys, chickens, goats, and humans[17]. Snakes appear to be important carriers of this bacterium, with as many as 78.8% harboring the organism[14].

Almost all infections from *Salmonella enterica* ssp. *arizonae* occur in patients with underlying medical conditions[16]. In 2005, Jeffry and Curtis stated, "serious infection has not been documented in a healthy human adult, although isolation of *Salmonella arizonae* should prompt evaluation of the immune status in an apparently healthy individual".

It has been observed that there is no standard therapy for *Salmonella arizonae* UTI, but the organism is susceptible to commonly prescribed antibiotics, including ampicillin and fluoroquinolones[15].

This study reports the involvement of *Salmonella arizonae* and other bacterial pathogens in human UTI and their susceptibility to commonly prescribed antibiotics.

MATERIALS & METHODS

Approval to conduct the study was obtained from the ethics committee of Specialist Hospital Sokoto (SHS) and informed consent was obtained from each participant. The study was conducted at the Microbiology Department Laboratory of SHS. SHS is a 300-bed referral hospital with some modern equipment and facilities. It provides healthcare services and is located in the capital city of the state of Sokoto in Northwestern Nigeria.

Collection of Clinical Isolates

A total of 365 non-repetitive urine samples were collected over a period of four months from patients (aged 18 years and above) visiting the general outpatient clinic (GOPC) of SHS; these included both male and female patients. Patients on admission, pregnant women and those coming for routine check-ups were excluded from the study. Early morning mid-stream clean catch urine samples were collected (by patients) in sterile disposable containers with screw caps. Prior to urine collection, patients were counseled on taking aseptic measures/precautions while collecting samples to avoid contamination.

Urine samples were inoculated on CLED agar using calibrated wire loop and incubated under aerobic conditions for 18–24 hours at 37°C. Pure cultures of individual isolates were obtained by sub-culturing on nutrient agar. Thereafter, agar slants of the pure culture were prepared for further analysis.

Identification of Bacteria Isolates

An 18–24-hour-old pure culture of the bacterial isolate to be identified was done. Oxidase test was carried out on the isolate prior to strip inoculation. Only oxidase negative isolates were considered. A loopful was emulsified from an 18–24-hour culture in 3 ml sterile 0.9% saline for the GN A microwell strip and was mixed thoroughly. Using a sterile Pasteur pipette, 3–4 drops (approximately 100 µl) of the bacterial suspension were added to each well of the strip(s). The GN A microwell strips were read after 18–24 hours of incubation at 37°C.

Note: *GN A is a type of Microgen GN-ID system which employs standardized biochemical substances in microwells to identify the family Enterobacteriaceae and other non-fastidious Gram-negative bacilli. GN A is intended for the identification of oxidase negative, nitrate positive, glucose fermenters constituting the most commonly occurring genera of the family Enterobacteriaceae.*

Antibiotic Susceptibility Testing (AST)

The susceptibility of isolates to seven commonly prescribed antibiotics was tested in SHS using the modified Kirby Bauer disc agar diffusion technique. The discs (Oxoid, UK) were ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), gentamicin (CN, 30 µg), nalidixic acid (NA, 30 µg), cotrimoxazole (SXT, 25 µg), nitrofurantoin (F, 300 µg), and amoxicillin/clavulanic acid (AMC, 30 µg).

Results were interpreted according to the CLSI guidelines of 2012.

RESULTS

Of the 365 urine samples analyzed over a four-month period to detect the presence of bacterial

pathogens, 61 (16.71%) were found to be Gram-negative isolates comprising the following: 19 (31.15%) *E. coli*, 15 (24.59%) *Salmonella arizonae*, 7 (11.48%) *Klebsiella pneumoniae*, 3 (4.92%) *Klebsiella oxytoca*, 6 (9.84%) *Enterobacter gergoviae*, 4 (6.56%) *Citrobacter freundii,* 4 (6.56%) *Serratia marcescens,* and 1 (1.64%) each of *Enterobacter aerogenes, Proteus mirabilis,* and *Edwardsiella tarda*. The results are shown in Table 1.

Table 1. Frequency of occurrence of isolates

Organism	Frequency of occurrence	% Frequency of occurrence
E. coli	19	31.15
Salmonella arizonae	15	24.59
Klebsiella pneumoniae	7	11.48
Enterobacter gergoviae	6	9.84
Citrobacter freundii	4	6.56
Serratia marscescens	4	6.56
Klebsiella oxytoca	3	4.92
Enterobacter aerogenes	1	1.64
Proteus mirabilis	1	1.64
Edwardsiella tarda	1	1.64

The result of the antibiotic susceptibility test revealed that *Salmonella arizonae* showed high resistance to commonly used antibiotics. These isolates showed the highest degree of resistance to nalidixic acid (13, 86.67%), followed by cotrimoxazole (12, 80%), gentamicin (10, 66.67%), norfloxacin (9, 60%), and amoxicillin/clavulanic acid (8, 53.33%). They showed the least resistance to ciprofloxacin (3, 20%) and nitrofurantoin (3, 20%), indicating enhanced sensitivity to these compounds. The result is presented in Table 2.

Table 2. Antibiotic resistant pattern of *Salmonella arizonae* against commonly used antibiotics

Antibiotics	No. of resistant isolate	% of resistant isolate
Ciprofloxacin	3	20
Norfloxacin	9	60
Gentamicin	10	66.67
Nalidixic acid	13	86.67
Cotrimoxazole	12	80
Nitrofurantoin	3	20
Amoxicillin/Clavulanic	8	53.33

DISCUSSION

The spectrum of uropathogens isolated from our study is not different from that reported in many studies. According to most studies conducted in Nigeria, *E. coli* was found to be the most prevalent Gram-negative uropathogen. In this study, *E. coli* was found to be the most prevalent Gram-negative bacteria. This is consistent with the results of other studies[3, 18–24]. Also, *E. coli's* emergence as the most common cause of UTI may be due to certain virulence factors such as hemolysin production and presence of fimbriae[25].

Salmonella arizonae had the second highest prevalence (23.44%). This is contrary to the results of a study that says *Salmonella arizonae* is an uncommon human pathogen with serious infections reported in immunocompromised hosts[16]. Although study on the prevalence of *Salmonella arizonae* in Sokoto is scarce and to some extent not available, Akinyemi *et al.,* in a study in 2007, reported a prevalence rate of 41.4% and 16.7% of *Salmonella arizonae* in stool and blood, respectively, in Lagos State, Nigeria[26].

About 11 isolates of *Salmonella arizonae* showed resistance against at least four antibiotics, indicating strong selective pressure. Ciprofloxacin and nitrofurantoin were the most effective antibiotics against *Salmonella arizonae.* The high resistance to nalidixic acid, cotrimoxazole, gentamicin and norfloxacin is consistent with the findings of certain reports that indicate extensive use of these agents (as they are most commonly prescribed, cheaper and easily available in hospitals and community pharmacies) has made *Salmonella arizonae* immune to the compounds[2, 13].

The findings revealed that Gram-negative isolates displayed various levels of resistance to the most frequently prescribed antibiotics in empirical treatment of UTIs. All Gram-negative isolates showed high susceptibility to nitrofurantoin, whereas susceptibility to cotrimoxazole was low. A study by Wariso *et al.* in 2010 in South-South Nigeria showed that the susceptibility of all uropathogens to

cotrimoxazole was 7.1%. Dada-Adegbola and Mulli also reported a lower susceptibility of 5.1% to cotrimoxazole in 2010. Accurate knowledge of local epidemiology and patterns of antimicrobial resistance in uropathogens is essential to design an effective and efficient therapy for UTIs[8].

CONCLUSION

Our study was conducted to create awareness about the prevalence of *Salmonella arizonae* as one of the major causes of UTIs. Our study demonstrated a high prevalence of *Salmonella arizonae* in Sokoto metropolis. Therefore, there is a need to look out for *Salmonella arizonae* to determine the clinical significance in UTI in this environment.

Note: *Our conclusion in this report is based on the high incidence of 15 (and not 7) of Salmonella arizonae.*

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