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Survival and Antimicrobial Resistance of *Salmonella enterica* isolated from Bathrooms and Toilets following Salmonellosis in Some Homes in Ado-Ekiti.

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Abstract: The survival of *Salmonella enterica* in different sites of bathrooms and toilets in fifty (50) domestic homes in Ado-Ekiti was investigated. The total percentage isolate from toilets was 44%; the highest been from the bowl water (70%) while in the bathroom it was 26% the highest been from sink (44%). The antimicrobial drug susceptibility of forty-four (44) of the isolates against ten (10) different conventional antibiotics was tested. The highest resistance was observed in Nalidixic acid (82%) and Ciprofloxacin (70%). Eight (8) isolates from the bathrooms were resistant to Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Nalidixic acid, Trimethoprin-sulfamethoxazole and Tetracycline. All isolates were susceptible to Cefotaxime and Cefepime; five (5) isolates from the toilets were intermediately susceptible to Cefepime (MIC 16µg/mL). Studies of Salmonella cases showed that the bacterium persisted in household bathrooms and toilets for several weeks after individuals had recovered from symptoms of salmonellosis.

Key words: Antimicrobial resistance, Survival, *Salmonella enterica*, Salmonellosis

INTRODUCTION

Members of the bacterial genus *Salmonella* are among the major pathogens that cause Salmonellosis in humans. Most human *Salmonella* infections are thought to be associated with food borne transmission from contaminated animal-derived meat and dairy products [12]. Human infections are commonly caused by ingestion of food that has been contaminated by animal feces [10] Although >2,500 serovars of *S. enteric* have been identified, most human infections are caused by a limited number of serovars. S. serovar Typhimurium and Enteritidis are the most common causes of human salmonellosis worldwide, although other serovars have been reported to be more prevalent in some regions [9]. Adequate sanitary measures have led to a decrease in cases of typhoidal Salmonella infections in developed countries such as the USA where the incidence is low. However, in these countries, non-typhoidal *Salmonellosis* is common, and most of these cases are associated with outbreaks from contaminated meat, dairy products or by cross-contamination from foods contaminated with *Salmonella* [7,4]. In Nigeria, morbidity associated with illness due to *Salmonella* continues to be on the increase and in some cases, resulting in death.

Although, the primary cause of Salmonellosis is consumption of contaminated foods, there is the potential for secondary spread, from person-to-person and also to other foods. Person- to-person spread with family groups is often associated with poor personal hygiene but there is the opportunity of air-borne and surface–to-surface spread within the toilet and the bathroom, especially during the diarrhea phase. Despite improved public health, serious infections with *Salmonella* remain a major clinical and public health concern in Nigeria and worldwide. [1]. *S. enterica* are a leading cause of food borne disease worldwide [8]. In the United States, as many as 1.4 million cases of *S. enterica*- associated disease occur annually [11,6,4]. While usually self-limiting, salmonellosis may require antimicrobial drug treatment in infants, the elderly or immunocompromised persons. However, antimicrobial drug resistance has become increasingly common in *S. enterica*, which can complicate therapy [5].

There is little documented evidence to show if in homes where individuals have or have had salmonellosis, environmental contamination in toilets and bathrooms contributed to the spread of the infection. This work intends to determine the survival of *Salmonella enterica* in bathrooms and toilets, locate the area where the bacteria can survive most, determine the necessary preventive measures and the resistance profile of the isolates to conventional antimicrobial drugs.

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MATERIALS AND METHODS

Collection of Samples: Environmental sampling was carried out in fifty (50) different domestic homes at Adebayo area of Ado-Ekiti where there were reported and confirmed cases of Salmonellosis. After proper consultation and permission granted, swabs were collected from surfaces of toilets and bathrooms with sterile swab sticks. Swabs were collected from recess under the rim of toilet bowl, toilet seat, toilet floor, toilet walls and flush handle and from the window platforms, doors and walls of bathrooms. The swabs were stored inside swab-stick sterile bottles containing 5ml of Selenite F broth and transferred to the laboratory.

Culture, Isolation and Identification of Samples: Samples were cultured on Salmonella-Shigella agar, incubated at 37^0C for 24hrs. Smooth colonies with black centre were sub cultured on to MacConkey agar (Oxoid), incubated at 37^oC for 18-24hr. Pale colonies were isolated and confirmatory biochemical test (Gram's reaction, urease reaction, citrate utilization and motility) were conducted. *Salmonella enterica* was identified by using standard biochemical tests and commercial typing antiserum (Statens Serum Institute, Copenhagen, Denmark) according to the manufacturer's instructions.

Antimicrobial Susceptibility profile: The susceptibility profile of forty-four (44) of the isolates was determined. Minimum inhibitory concentration (MIC's) of ten (10) antimicrobial drugs: **Phenols** (chloramphenicol), **Penicillins** (Ampicillin), **Cephalosporin (**cefepime, cefotaxime), **Tetracycline** (tetracycline), **Aminoglycosides** (gentamicine, streptomycine), **Sulfonamides** (trimethoprin-sulfamethoxazole), **Quinolones and Fluoroquinolone** (nalixidic acid, ciprofloxacin) were determined by using the broth- microdilution method; susceptibility to streptomycin was measured by using the disk-diffusion method as recommended by the Clinical and Laboratory Standards Institute[3].

RESULTS AND DISCUSSION

The percentage isolation of *Salmonella enterica* from toilets is shown in Table 1. At different sites of the toilet such as seats 14 (28%), bowl 23(46%), bowl water 35(70%), recess under rim 27(54%) and flush handle 8(16%) were estimated. The percentage isolation of the bacterium from bathrooms is shown in Table 2. The walls 11(22%), sink 22(44%), window 8(16%), floor 19(38%) and door 6(12%) showed these percentages respectively. While the toilets have a total percentage isolation of 111 (44%) the bathroom have a total percentage isolation of 66 (26%). The predominance of *S. enterica* in the bowl of toilets has earlier been reported by Barker and Wheeler [2]. It was reported that the toilet can be a reservoir of large numbers of microorganisms, particularly in wet areas. In homes were a family member had Salmonellosis, four out of six toilets tested positive for Salmonella under the recess of the toilet bowl rim [2]. The percentage of isolates from toilets and bathrooms that were resistant to antimicrobial drugs is given in Table 3. Of the 44 isolates, 36(82%) were resistant to nalidixic acid and 31(70%) were resistant to ciprofloxacin. Only 3 isolates, recovered in the bathrooms were susceptible to all 10 tested antimicrobial drugs; 36(82%) displayed resistant to at least three (3) drugs. Of the eight (8) antimicrobial drug-resistant isolates identified, most was resistant to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, trimethoprin-sulfamethoxazole and tetracycline. All isolates were susceptible to cefotaxime and cefepime; five (5) isolates obtained in the toilets were intermediately susceptible to cefepime (MIC 16μg/mL).

Studies of Salmonella cases showed that the bacterium persisted in household bathrooms and toilets for several weeks after individuals had recovered from symptoms of salmonellosis. It was observed that the toilet bowl water and the bathroom sink harbors the largest number of the bacteria. The domestic toilet harbors the largest percentage of *Salmonella enterica.* The highest resistance observed in tetracycline, trimethoprin-sulfamethoxazole nalidixic acid, ampicillin, and ciprofloxacin in this study may be due to several factors. Firstly patients who had salmonellosis were before isolation undergone antimicrobial therapy which may be inappropriately used. Though there was no antimicrobial drug-use information, the easy access to antimicrobial drugs raises the possibilities that the patients might have taken drugs after the onset of the illness. The findings of this work reveal that during and after salmonellosis, there is considerable risk of spread of antimicrobial multi- drug resistant bacteria to other family members via the environment including direct shedding of skin, contaminated hands and surfaces in the bathrooms and toilets.

We hope to promote greater awareness on strong link between antimicrobial drug use and the development of resistance and to make clear that improving hygiene both in bathrooms and toilets is of paramount importance especially after an apparent recovery of a salmonellosis patient.

Table 1: Percentage isolation of *Salmonella enterica* from toilets (n=50)

Sample sites	N (%)	
	Detected	Not detected
Seat	14(28)	36(72)
Bowl film	23(46)	27(54)
Bowl water	37(70)	15(30)
Recess under rim	27(54)	23(46)
Flush handle	8(16)	42(84)

Table 2: Percentage isolation of *Salmonella enterica* from bathroom (n=50)

Sample sites	N (%)	
	Detected	Not detected
Walls	11(22)	39(78)
Sink	22(44)	28(56)
Window	8(16)	42(84)
Floor	19(38)	31(62)
Door	6(12)	44(88)

Table 3: Antimicrobial Drug Susceptibility Profile

Antimicrobial	MIC µg/mL	No resistant isolates
Agents.		n = 44(%)
Chloramphenicol	≥32	33(75)
Ampicillin	≥32	35(78)
Cefepime	≥16	0 (0)
Cefotaxime	≥16	0 (0)
Tetracycline	≥16	36(82)
Gentamicin	≥16	35(78)
Streptomycin	ND	40(91)
Trimethoprin-Sulfa	≥4/76	36(82)
Nalidixic acid	≥32	36(82)
Ciprofloxacin	≥4	31(74)

ND- Not determined

REFRENCES

1. Aarestrup, F.M., M. Lertworapreecha, M.C. Evans, A. Bangtrakulnonth, T. Chalermchaikit and R.S. Hendriksen, 2003. Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar Weltevreden from different countries. J. Antimicrob Chemother., 52: 715-718.
2. Barker, J. and J.G. Wheeler, 1999. Hygiene in the domestic setting: The International Situation., 57: 189-204.
3. Clinical and Laboratory Standard Institute, 2007. Performance standards for Antimicrobial susceptibility testing: Seventeenth Information Supplement Vol 27. Wayne (PA): The committee: Document, No. M-100-S17.
4. Egorovo, S., L. Kaftyreva, P.A.D. Grimont and F.X. Weill, 2007. Prevalence and characterization of extended- spectrum cephalosporin-resistant nontyphoidal Salmonella isolates in adults in Saint Petersburg, Russia (2002-2005). Microb. Drug. Resist., 13: 102-107.
5. Egorovo, S., M. Timinouni, M. Demartin, S.A. Granier, J.M. Whichard, V. Sangal, L. Fabre, A. Delaune, M. Pardos and Y. Millemann, 2008. Ceftriaxone- resistant *Salmonella enterica* Serotype Newport, France. Emerg. Infect. Dis., 14(6): 954-957.
6. Galanis, E., D.M. Lo Fo Wong, M.F. Patrick, N. Binsztein, A. Cieslik, T. Chalermchikit, 2006. Web-base surveillance and global Salmonella distribution, 2000-2002 Emerg. Infect. Dis., 12: 381-.

7. Glynn, M.K., J.G. Wright, C. Bopp, 2005. Emergence of multidrug resistance in *Salmonella enterica* serotype *typhimurium* DT 104 infections in the United States. Nat. Engl. J. Med., 338: 1333-1338.
8. Hald, T., D.M. Lo Fo Wong and F.M. Aarestrup, 2007. The attribututtion of human infection with antimicrobial resistant Salmonella bacteria in Denmark to sources of animal origin. Foodborne Pathog Dis., 4: 313-326.
9. Herikstad, H., Y. Motarjemi, R.V. Tauxe, 2002. Salmonella surveillance a global survey of public health serotyping. Epidermiol. Infect., 129: 1-8.
10. Humphery, T.J., 2000. Public health aspects of Salmonella infections. Walling-Ford (England): CABI Publishing.
11. Voetsch, A.C., T.J. VanGilder, M.M. Farley, S. Shallow, R. Marcus, 2004. Food Net estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin. Infect Dis., 3: 127-134.
12. Winokur, P.L., 2003. Molecular epidemiological techniques for Salmonella strain discrimination. Front Biosci., 8: c14-24.