International Journal of Science, Environment and Technology, Vol. 2, No 5, 2013, 877 – 885

ISSN 2278-3687 (O)

PREVALENCE OF *YERSINIA ENTEROCOLITICA* IN HUMAN POPULATION AND TRANSMISSION VEHICLES IN ANAMBRA STATE, NIGERIA

***Amasiani, R.N.[1], Agbo, I.C.[2], Ezeifeka, G.O.[3] and Chah, K.F.[2]**

[1]Nnamdi Azikiwe University, Awka,

[2]Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka.

[3]Department of Veterinary Microbiology and Parasitology,

Michael Okpara University of Agriculture, Umudike

E-mail: amasianirosenwanneka@yahoo.com (**Corresponding Author*)

Abstract: Two hundred and ninety-two stool samples from diarrheic and non-diarrheic patients and 208 samples from different transmission vehicles such as ice-cream, sachet water, stream water, borehole water, rain water, well water and beef suya from the six health zones of Anambra State were screened for *Yersinia enterocolitica*. The isolation method used was cold enrichment using phosphate buffered saline prior to subculturing onto cefsulodin-irgasan-novobiocin (CIN) agar. Pathogenicity testing and antimicrobial susceptibility testing on recovered isolate were performed. Of the 292 human faecal samples screened, 75 (25.7%) were positive for *Y. enterocolitica*, while 37 (17.8%) of the 208 possible transmission vehicles analyzed yielded growth of *Y. enterocolitica*. The prevalence of *Y. enterocolitica* infection was highest among individuals aged between 1 – 10 years (36.1%) while those 41 – 50 years and 71 – 80 years did not harbor the organisms. None of the isolates was resistant to ciprofloxacin, ofloxacin, gentamicin and tetracycline while 97.3% were resistant to amoxicillin-clavulanic acid. All isolates were resistant to co-trimoxazole and amoxicillin.

Keywords: *Yersinia enterocolitica*, human stool, transmission vehicle, Nigeria.

INTRODUCTION

Yersinia enterocolitica is a pleomorphic Gram-negative bacillus that belongs to the family Enterobacteriaceae. It is a well described enteric pathogen with distinctive clinical manifestations, a range of outcomes and predilection for children (Bottone, 1999). Although such infection occurs at all ages, the majority of patients are usually less than 5 years old (Koehler *et al*., 2006). The spectrum of the disease ranges from asymptomatic to life threatening sepsis especially in infants.

The incidence of human infection caused by *Y. enterocolitica* has increased tremendously with reports coming from several parts of the globe (Schlundt, 2002; Bottone, 1999). In Nigeria it has been incriminated in human clinical cases and has been isolated from

*Received Aug 11, 2013 * Published October 2, 2013 * www.ijset.net*

apparently healthy animals (Okwori *et al*., 2009). Strains of *Y. enterocolitica* have been isolated from a wide variety of foods as well as from animals and environmental sources such as water (Sandery *et al*., 1996), milk and milk products (Okwori *et al*., 2009), vegetables (Cocolin and Comi), meats (Fredriksson-Ahomaa *et al*. 2006, Grahek-Ogden *et al*., 2007), animals and human beings (Fredriksson-Ahomaa *et al*., 2000, Fredriksson-Ahomaa *et al*. 2007).

Most cases of *Y. enterocolitica* infection are sporadic but reports have documented large outbreaks centred on a single contaminated source (Zartash, 2009). *Yersinia enterocolitica* is associated with acute diarrhea, terminal ileitis, mesenteric lymphadenitis and pseudo-appendicitis (De Berardis *et al*., 2004). *Y. enterocolitica* has caused high rate of morbidity and mortality globally among children as a result of poor hygiene and lack of access to portable water. Its incidence has been reported in different parts of West Africa including Nigeria (Okwori *et al*., 2009).

In Nigeria, much attention has not been given to the routine isolation of this pathogenic organism which also is a major cause of acute gastroenteritis in patients especially children. There is paucity of information on the distribution and pattern of occurrence of the organism in our environment. There is need to identify the risk factors that predispose to the occurrence of the organism in various clinical hosts and reservoirs. Therefore, the objective of this study was to determine the prevalence and risk factors of *Y. enterocolitica* in human population in Anambra State, Nigeria.

MATERIALS AND METHOD

Study area and sampling method

This study was conducted in Anambra State, Nigeria. The State is divided into six health zones. Two hospitals from each health zone were selected using simple random technique.

Isolation and identification of *Y. enterocolitica*

A total of 500 samples, comprised of 292 feacal samples from diarrheic and non diarrheic hospital patients and 208 samples from transmission vehicles such as milk and ice-cream, water (stream, boreholes, well water, rain water and sachet water), (beef suya were randomly collected from the six zones in Anambra State. Two grams of each faecal sample were suspended in 10 ml of phosphate buffered saline (PBS) (pH 7.2), vortexed for 30 s and subjected to cold enrichment by incubating at 4^0C for 21 days. A unit volume of each transmission vehicle (except beef suya) was added to 9 ml of PBS (pH 7.2), mixed

thoroughly and also subjected to cold enrichment. For suya samples, 2 g of each sample was pounded in a mortar then suspended in 9 ml of PBS (pH 7.2), mixed thoroughly and similarly incubated. After cold enrichment a loopful of each sample was streaked on Cefsulodin-Irgasan-Novobiocin (CIN) agar (Oxoid, Basingstoke, United Kingdom). Inoculated plates were incubated at 25^0C for 24-48 hrs. Two to three colonies with deep red/purple centres and sharp edges surrounded by a translucent boarder ("bull eye") were selected and purified on fresh CIN agar for Gram staining and motility test (Cheesebrough, 2004). Presumption *Yersinia* colonies were biochemically confirmed by catalase, coagulase, Indole, Methyl-red, Voges- Proskauer (IMViC) and sugar (rhamnose, sucrose and sorbitol) fermentation tests (Cheesebrough, 2004).

Pathogenicity testing on mice

Swiss albino mice were used to determine the invasive ability of *Y. enterocolitica*. The effects on mice mimic the major pathological feature of human disease. The mice were infected by oral administration (peroral test), intraperitoneal and eye inoculation (Sereny test) as described by Ali and Shareef (1991).

Per-oral test: A colony of the test organism was suspended in 1ml of normal saline. Three Swiss albino mice were deprived of drinking water for more than 12 hrs and were allowed to drink the above suspension. Death of two or more mice was considered a positive per-oral result according to Ali and Shareef (1991).

Intra-peritoneal test: A colony of the test organism that were grown at 25 ^{0}C for 24 – 48hrs on CIN agar was suspended in 1ml normal saline. 0.1 ml of the suspension was injected intra-peritoneally into each of three albino mice. Death of the mice between 2 – 7 days was considered a positive result according to Ali and Shareef (1991).

Sereny test: The eyes of three albino mice were infected with one loopful of the organism that was suspended in 1ml of normal saline. Development of conjunctivitis gives a positive sereny test as described by Ali and Shareef (1991).

Antimicrobial susceptibility testing

The sensitivity profile of each isolate to seven antibiotics (ciprofloxacin [10 µg], ofloxacin [10 µg], gentamicin [10 µg] tetracycline [25 µg], amoxicillin-clavulanic acid [25 µg], amoxicillin [25 µg] and cotrimoxazole [25 µg]) was performed using the standard disc diffusion method (Konemman *et al*., 2006). The diameter of inhibition zones produced by the different antibiotics against each test organism was measured using a meter rule (CLSI, 2008).

RESULTS

The prevalence and distribution of *Y. enterocolitica* in humans and transmission vehicles in Anambra State is shown in Table 1. Of the 292 human faecal samples screened, 75 (25.7%) were positive for *Y. enterocolitica*, while 37 (17.8%) of the 208 possible transmission vehicles analyzed yielded growth of *Y. enterocolitica*. Beef suya (63.3%), stream water (38.5%) and ice-cream (26.8%) were the transmission vehicles found to be contaminated by *Y. enterocolitica* (Table 1). The prevalence of *Y. enterocolitica* infection was highest among individuals aged between 1 – 10 years (36.1%) while those 41 – 50 years and 71 – 80 years did not harbor the organisms (Table 2).

Out of the 75 isolates from human samples 50 (66.7%) caused death in mice 2 – 7 days following intra-peritoneal or oral administration as well as conjunctivitis in the Sereny test. Forty-seven of the 50 isolates pathogenic to mice were isolated from diarrheic patients. None of 37 isolates from transmission vehicles was pathogenic to mice.

The antibacterial resistance profile of the isolates is presented in Table 3. None of the isolates was resistant to ciprofloxacin, ofloxacin, gentamicin and tetracycline while 97.3% were resistant to amoxicillin-clavulanic acid. All isolates were resistant to cotrimoxazole and amoxicillin.

DISCUSSION

The prevalence of *Y. enterocolitica* (36.1%) recorded among children between 1 – 10 years of age in this study is similar to the findings of Omoigberale and Abiodun (2002) who documented a prevalence rate of 32.8% among diarrheic children in Benin, Nigeria. The isolation rate in the present study, however, differed from 7.5% and 1.4% reported in the same age group by Okwori *et al*. (2007) in Jos and Onyemelukwe (1993) in Enugu respectively. Nigeria had generally reported low prevalence of *Y. enterocolitica* infection compared to other parts of the world. In Southeast, United States of America (between 1988 – 1991) 77.6% of infections in children aged 12 months and younger were caused by *Y. enterocolitica,* making the organism the second most common cause of bacterial gastrointestinal infection in children (Metchock *et al*., 1991)

The high prevalence of *Y. enterocolitica* infection as seen amongst children 1 – 10 years of age could be due to impaired or compromised immunity, social and sanitary habits as documented in a similar finding by Lal *et al*. (2003). In this study, it was observed that

prevalence of *Y. enterocolitica* decreased as the age increased. This may result from improved personal hygiene as the individuals attain adulthood.

Yersinia enterocolitica is ubiquitous in the natural environment and may have originated from water, soil, and food contamination. It was not isolated from industrial ice cream but rather from non-industrial ice cream. Francis *et al*., (1980) found out that *Y. enterocolitica* does not survive pasteurization. However, other worker reported that viable *Y. enterocolitica* may persist after pasteurization if the organism is present in large numbers (Ackers *et al*., 2000, Salmah and Shareef, 1991). These finding may explain the absence of *Y. enterocolitica* from industrial ice cream. The absence of *Y. enterocolitica* in the sachet water, borehole water and rain water could be due to boiling during processing of sachet water, chlorination of sachet and borehole water and lack of exposure of the three to any subsequent animal or human contamination.

As mentioned previously, *Y. enterocolitica* is an ubiquitous microorganism and may be expected to be found in many foods. In the present investigation 37 *Y. enterocolitica* isolates were obtained from ice cream, stream water, well water and beef 'suya'. The prevalence rate of 25.7% indicates that the probable source of human infection was through these transmission vehicles. In addition, *Y. enterocolitica* has been isolated from samples of ingredients used in the production of ice-cream such as cream, egg and pasteurized milk (Tacket *et al*, 1994). Norma and Ana, (2000) also reported an outbreak of yersiniosis associated with consumption of contaminated water and food.

The effect in mice mimics the major pathological features of human disease by causing deaths of mice within 2-7 days in intraperitoneal and oral administration and conjunctivitis in sereny test (Aulisio *et al*., 1983). Une (1977) demonstrated that pathogenic strains of *Y. enterocolitica* were able to penetrate cultured HeLa cells, whilst non-pathogenic strains could not. Recovery of the test isolates from vital organs, stool sample and pus from the eyes of the infected mice used for animal model proves the invasiveness of the organism, hence its pathogenicity.

In this study, antimicrobial profile of *Y. enterocolitica* to different antibiotics showed that all the isolates were susceptible to ciprofloxacin, ofloxacin, gentamicin and tetracycline. Only three isolates were susceptible to amoxicillin-clavulanic acid. Strains isolated by Okwori *et al*. (2007), in Jos, Nigeria were susceptible to ciprofloxacin, floxavid and streptomycin.

In conclusion, this study has shown that *Y. enterocolitica* is present and active in human population and some transmission vehicles in Anambra State, Nigeria. However it may go

undetected because of ineffective isolation procedures or because of its similarity to other enterobacteria with respect to biochemical reactions. Efforts should be made to include the isolation of this organism in our routine laboratory exercises to reduce death that might occur when the organism is implicated in human disease.

REFERENCES

[1] Ackers ML, Schoenfeld S, Markman J. An outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk. J Infect Dis 2000; 181: 1834–1837.

[2] Ali AS, Shareef EM. Incidence of *Yersinia enterocolitica* in some food and environmental samples in Saudi Arabia. J King Saud Univ 1991; 3: 91 – 100.

[3] Aulisio CCG, Hull WE, Stanfield JT, Sellers RC. Evaluation of virulence factor testing and characteristics of pathogenicity of *Yersinia enterocolitica*. J Infect Immunol 1983; 40: 330 – 335.

[4] Bottone EJ. *Yersinia enterocolitica*: overview and epidemiologic correlates. Microbiol Infect 1999; 1: 323–333.

[5] Cocolin L, Comi G. Use of a culture-independent molecular method to study the ecology of *Yersinia* spp. in food. Intl J Food Microbiol 2005; 105: 71–82.

[6] Cheesebrough, M. District laboratory practice in tropical countries Part 2. Cambridge University Press 2004; 125 – 267.

[7] Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial disk and diffusion susceptibility tests for bacteria isolates from animals: Approved standards – 3rd edition 2008; 28: M31- A3.

[8] De Berardis B, Torresini G, Brucchi M, Marinelli S, Mattucci S, Schietroma M, Vecchio L, Carlei F. *Yersinia enterocolitica* intestinal infection with ileum perforation: report of a clinical observation. Acta Biomed 2004; 75: 77-81.

[9] Francis DW, Spaulding PL, Lovett J. Exotoxin production and thermal resistance of *Yersinia enterocolitica* in milk. Appl Environ Microbiol 1980; 40: 174 – 176.

[10] Fredriksson-Ahomaa M, Bj¨orkroth J, Hielm S, Korkeala H. Prevalence and characterization of pathogenic *Yersinia enterocolitica* in pig tonsils from different slaughterhouses. Food Microbiol 2000; 17: 93–101.

[11] Fredriksson-Ahomaa M, Stolle A, Korkeala H. Molecular epidemiology of *Yersinia enterocolitica* infections. FEMS Immunol Med Microbiol 2006; 47: 315–329.

[12] Fredriksson-Ahomaa M, Stolle A, Stephan R. Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. Intl J Food Microbiol 2007; 119: 207–212.

[13] Grahek-Ogden D, Schimmer B, Cudjoe KS, Nygard K, Kapperud G. Outbreak of *Yersinia enterocolitica* serogroup O:9 infection and processed pork, Norway. Emerg Infect Dis 2007; 13: 754–756.

[14] Koehler KM, Lasky T, Fein SB. Population-based incidence of infection with selected bacterial enteric pathogens in children younger than five years of age, 1996-1998. Pediatr Infect Dis J 2006; 25: 129-134.

[15] Konemman EW, Washington WC, Stephen DA, William MJ, Garry WP, Paul CS, Gaul LW. Color Atlas and Textbook of Diagnostic Microbiology, 6th Edition. Lippincott William and Wilkins, New York 2006; 367: 991 – 992.

[16] Lal M, Kaur H, Gupta LK. *Yersinia enterocolitica* Gastroenteritis – A prospective study. Indian J Med Microbiol 2003; 21: 186-188.

[17] Metchock B, Lonsway DR, Carter GP, Lee LA, Macgowon JE. *Yersinia enterocolitica*: A frequent seasonal stool isolate from children at an urban hospital in the southern United States. J Clin Microbiol 1991; 29: 2868 – 2869.

[18] Norma AB, Ana M. Isolation of *Yersinia enterocolitica* in ice-cream at different pH stored at -18^{0}C. Brazilian J Microbiol 2000; 31: 174 – 177.

[19] Okwori AEJ, Agada GAO, Olabode AO, Agina SE, Okpe ES, Okopi J. The prevalence of pathogenic *Yersinia enterocolitica* among diarrhea patients in Jos, Nigeria. African J Microbiol 2007; 6: 1031 – 1034.

[20] Okwori AEJ, Mart´ınez PO, Fredriksson-Ahomaa M, Agina SE, Korkeala H. Pathogenic *Yersinia enterocolitica* 2/O:9 and *Yersinia pseudotuberculosis* 1/O:1 strains isolated from human and non-human sources in the Plateau State of Nigeria. Food Microbiol 2009; 26: 872–875.

[21] Omoigberale AI, Abiodun PO. Prevalence of *Yersinia enterocolitica* among diarrheal patients attending University of Benin Teaching Hospital Benin-City Nigeria. Sahel Med J 2002; 5:182-185.

[22] Onyemelukwe NF. *Yersinia enterocolitica* as an aetiological agent of childhood diarrhea in Enugu Nigeria. Central African J Med 1993; 39:192-195.

[23] Salmah AUA, Shareef EM. Incidence of *Yersinia enterocolitica* in some food and environmental samples in Saudi Arabia. J King Saud Univ 1991; 3: 91 – 100.

[24] Sandery M, Stinear T, Kaucner C. Detection of pathogenic *Yersinia enterocolitica* in environmental waters by PCR. J Appl Bacteriol 1996; 80: 327– 332

[25] Schlundt J. New directions in foodborne disease prevention. Intl J Food Microbiol 2002; 78: 3–17.

[26] Tacket CO, Narain JP, Sattin R, Lofgrn JP, Konigsberg C, Rendtorff RCJ, Rausa A, *et al*. A multi-state outbreak of infections caused by *Yersinia enterocolitica* transmitted by pasteurized milk. J America Med Assoc 1994; 251: 483 -486.

[27] Une T. Studies of on the pathogenicity of *Yersinia enterocolitica* experimental infection in rabbits. J Infect Immun 1977; 21: 449 – 363.

[28] Zartarsh Zafar Khan MB. *Yersina enterocolitica*. Fellow in Infectious disease, University of Oklahoma Health Science Center 2009; 1 – 12.

Tables

Table 1: Prevalence of *Y. enterocolitica* in humans and transmission vehicles in Anambra State

Sample source	**Number screened**	**No (%) positive**
Humans		
Diarrheic	87	65 (74.7)
Non-diarrheic	205	10 (4.9)
Total	**292**	**75 (25.7)**
Transmission vehicle		
Ice cream	41	11 (26.8)
Stream	39	15 (38.5)
Sachet water	25	0 (0.0)
Borehole water	35	0 (0.0)
Well water	45	2 (4.4)
Rain water	9	0 (0.0)
Beef suya	14	9 (63.3)
Total	**208**	**37 (17.8)**

Table 2: Percentage distribution of *Y. enterocolitica* isolates among different age groups

Age group	No. screened	No. (%) positive
1-10	97	35 (36.1)
11-20	76	20 (26.3)
21-30	39	10 (25.6)
31-40	37	7 (18.9)
41-50	13	0 (0.0)
51-60	11	2 (18.2)
61-70	17	1 (5.9)
71-80	2	0 (0.0)
Total	**292**	**75 (25.7)**

Table 3: Resistance profile of *Y. enterocolitica* isolates (n=112) to common antibacterial agents

Antibacterial agent	No. (%) of resistant isolates
Ciprofloxacin	0 (0.0)
Ofloxacin	0 (0.0)
Gentamicin	0 (0.0)
Tetracycline	0 (0.0)
amoxicillin-clavulanic acid	109 (97.3)
Cotrimoxazole	112 (100.0)
Amoxycilin	112 (100.0)