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Full Length Research

The prevalence of pathogenic *Yersinia enterocolitica* among diarrhea patients in Jos, Nigeria

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One hundred and fifty (150) stool samples from diarrhoeic children and adults seeking for medical attention (including hospitalized patients) in Vom Christain Hospital (VCH), Mandela Clinic (MC) Vom and Dagott Family Health Clinic (DFHC) Vom were screened for *Yersinia enterocolitica* infection between August 2005 and August 2006. The isolation methods adopted were direct plating on MacConkey Agar (MCA), Deoxycholate Citrate Agar (DCA) and cold enrichment method using phosphate buffered saline prior to subculture onto selective solid culture media (Cefsulodin Irgasan Novobiocin [CIN] agar). Out of the 150 samples screened, 6 (15%) were positive. The incidence of the infection was highest among those aged 1 - 10 years 3 (7.5%), followed by 21 - 31 years 2 (5%) and 11 - 20 years 1 (2.5%). Serotyped and biotyped, pathogenic *Y. enterocolitica* (2/O: 9. 4/O: 9) were susceptible to ciprofloxin, floxavid, streptomycin and tetracycline.

Key words: Diarrhea, *Yersinia enterocolitica*, Nigeria.

INTRODUCTION

Yersinia enterocolitica is emerging world wide as an enteric pathogen responsible for a wide spectrum of clinical manifestations including acute gastroenteritis (Ray et al., 2004), messenteric lymph adenitis, endocarpditis (Karachalios et al., 2002) predominantly affecting young children and has been known as the major cause of diarrhea in most of the industrialized world (Bottone and Robbin, 1997). *Y. enterocolitica* is thought to be a significant food borne pathogen even though pathogenic isolates have seldom been recoverd from foods (de Boer, 1995). The organism may be separated by serotyping into approximately 60 serogroups, of which only 11 serogroups are most frequently associated with human infections (with serogroups 0:3, 0:8, 0:9 and 0:5.27 predominating) (La Scola et al., 1997; Wannet et al., 2001). *Y. enterocolitica* strains that were the most common causes of yersniosis in Europe and Japan (Serotype 0:3, and 0:9) (Okamoto et al., 1983) were virtually unknown in the United States of America. However, the distinction between American and non-American strains seem to have faded (Bottone, 1999). Of the six biotypes of *Y. enterocolitica*, five (biotypes 1B, 2, 3, 4 and 5) are considered pathogenic in humans (Carniel, 2002). Strains of these pathogenic biotypes contain marker associated with virulence and these are located on the chromosome and on the (PYV) virulence plasmid (Goverde et al., 1993). *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 drinking water. Diarrheal diseases are major cause of children morbidity and mortality worldwide especially in developing countries (Ribeiro, 2000). This study was undertaken to determine the prevalence of *Y. enterocolitica* in children and adults presenting with diarrhea.

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MATERIALS AND METHODS

Sample collection

The diarrhoeic patients used in this study were drawn from Vom and its environment in Jos South L. G. A. of Plateau State. The samples were collected over 6 months period (August, 2005–August, 2006). A total of 150 faecal samples were screened.

Table 1. Percentage distribution of isolates from different age groups screened.

Age group	No. of sample	No. positive	Percentage positive
1-10	40	3	7.5
11-20	40	1	2.5
21-30	40	2	5.0
31-40	18	0	0
41-50	7	0	0
51-60	2	0	0
61-69	3	0	0
Total	**150**	**6**	**15.0**

(P<0.5)

Table 2. Phenotypic profiles of *Y. enterocolitica* strains.

Age group	No. of strains	Serotype	Biotype
1-10	3	O:9	2 (2)* 4 (1)*
11-20	1	O:9	2
21-30	2	O:9	2

*Number of strains

Cold enrichment

About 1 – 2 g of faecal sample was added to a tube containing 10 ml of phosphate buffered saline (pH 7.2), vortexed and homogenized for about 30 s and incubated at 4°C for three weeks and subsequently subcultured unto Deoxycholate Citrate Agar (DCA), MacConkey Agar (MCA) and Cefsulodin Irgasan Novobiocin Agar (CIN). The culture plates were incubated at 25 - 28°C for between 18 - 24 h (CFSAN, 2001).

Bacterial isolation and identification

Culture plates (DCA (Lab M, Lancashire, UK), MCA (Fluka, Sigma Aldrich Chemie, GmBH, Germany), CIN (Oxoid, UK) and the bacterial colonies were examined macroscopically and microscopically after incubation. Suspected colonies were further subjected to motility test by hanging drop technique both at 25 and 37°C. In addition, biochemical test (API 20E, Biomereux, France) including urease activity were used for the bacterial identification (Sharma et al., 1990).

Serotyping

Serological typing was done by slide agglutination test using specific typing sera O:1, O:2, O:3, O:5, O:8, O:9 for *Y. enterocolitica* (Denka Seiken, Japan).

Biotyping

Isolates were biotyped according to the revised scheme of Wauters et al. (1987) using pyrazinamidase activity, esculin hydrolysis, salicin acidification, tween-esterase activity, indole production, xylose acidification and nitrate reduction. All strains were recognised as pathogenic by virtue of their biochemical classification of Wauter et al. (1987).

Antimicrobial susceptibility

The sensitivity spectrum of each of the isolates to eight (8) different antibiotics was determined by standardized single disc diffusion method (Bauer et al., 1966).

Data management and analysis

Laboratory results were entered and managed using Microsoft Excel (windows 1997, Duxbury press). Descriptive statistics analysis was done using the program. The Kruskal-Wallis test was used for the comparison of results between individual groups of patients. Prevalence of *Y. enterocolitica* strains were compared among age groups.

RESULTS

Out of the 150 stool samples bacteriologically screened for enteric bacteria 6(15%) were positive for *Y. enterocolitica* (Table 1). Of the total isolates, the prevalence of *Y. enterocolitica* infection was highest among those aged 1 to 10 years (7.5%) followed by those aged 21 to 30 years (5.0%) (Table 2). The last group of patients within the age 31 to 69 years had no records of *Y. enterocolitica* infection.

All isolates of *Y. enterocolitica* were gram negative rods and motile at 25 - 28°C but non motile at 37°C. The colonies on CIN agar appeared as bull's eye surrounded by a transparent border (Lal et al., 2003) in contrast to those of other enteric bacteria most of which were pink to colourless in nature. All isolates were negative for oxidase and H_2S production but hydrolysed urea (Oxoid, UK).

Isolated strains of *Y. enterocolitica* gave positive react-

Table 3. Susceptibility testing of *Yersinia enterocolitica* to common antibiotics.

Antimicrobial drug	Disc potency (µg/ml)	Zone of inhibition (mm)	Susceptible isolates
Ciprofloxacin	10	30	6
Floxavid	20	25	6
Streptomycin	10	15	6
Chloramphenicol	10	0	0
Cloxacillin	10	0	0
Tetracyline	20	15	6
Ampicillin	20	0	0
Amoxycilin	30	0	0

ion to specific typing sera 0:9 and biotype 2. The drug sensitivity revealed that all the isolates were sensitive to ciprofloxacin, floxavid and streptomycin and tetracycline (Table 3). All *Y. enterocolitica* strains were found resistant to chloramphenicol, cloxacillin, ampicillin, amoxycillin (Abtek biologicals Ltd, UK).

DISCUSSION

Y. enterocolitica was considered a rare micro-organism for a longtime, but during the last decades it has been isolated all over the world from animals, raw food materials, environment, water and human being (kapperud, 1977; Ostroff, 1995; Singh et al., 2003; Okwori et al., 2005). The distribution of *Y. enterocolitica* in different age groups as obtained in this study was seen as a confirmation and an extension of the original observation of diarrhea due to *Y. enterocolitica* documented in some countries (Agbonlahor, 1986; Soltan-Dallal and Moezardalan, 2004; Adegunloye, 2006).

The prevalence of *Y. enterocolitica* (7.5%) recorded among children population of age between 1 - 10 years in this study is similar to the findings of Onyemelukwe (1993) who documented prevalent rate (1.4%) of *Y. enterocolitica* strains from faecal samples of children between the age groups of 1 - 12 in Enugu, Nigeria. However, our findings was much lower compared with the study of Omoigberale and Abiodun (2002) who in a similar study documented a prevalence rate of 32.8% among diarrhoeic children in Benin, Nigeria. Studies in Africa particularly Nigeria has revealed low prevalence of diarrhea due to *Y. enterocolitica* unlike other parts of the world especially Northern European Countries with a frequency of up to 13% (Ostroff et al., 1994; WHO, 1983).

The high prevalence of *Y. enterocolitica* as seen amongst children 1 - 9 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). Our findings incriminated *Y. enterocolitica* as a pathogen associated with diarrhea in this part of the world.

Diarrhea has been reported to occur among all age groups particularly in the developing countries and has been notably prevalent among children in the first two years of life (Patwari et al., 1993). During this study, it was observed that diarrhoeic adolescents aged between 11 - 20 years had a lower infection rate of 2.5% compared with 5.0% in adults aged 21 - 30 years. This is in agreement with the findings of Stolk-Engelaer and Konstainje (1996).

Most of the children screened were identified with poor culinary practices, low level of personal and environmental hygiene. Data derived from most hospitalized diarrhoeic children who tested positive for *Y. enterocolitica* showed that they were cared for by less educated nannies, private day care centres which were not properly equipped with standard facilities such as good toilets and clean water. This is in harmony with the previous findings by Adegunloye (2006). According to previous studies, the highest frequency of *Y. enterocolitica* was in cool weather rural areas, based on the presence of the most important sources of contamination such as pigs, cows, rabbits and dogs contaminating surfaces with their faeces (Zheng and Xie, 1996; Thiodeau et al., 1999). This is similar to our finding since most of the children who tested positive for *Y. enterocolitica* were being taken care of in homes where they had direct contact with dust, wastes and faeces of pet animals such as dogs and cats roaming within the premises. The main risk factors for the morbidity and mortality of diarrhea are well known and relate to a poor quality of life, lack of sanitation and clean water supply for most of the population living in poor areas of developing countries (Gonul and karapinar, 1991). Despite the fact that *Y. enterocolitica* is an important cause of diarrhea in some European and Scandinavian countries with cold climate, this study has emphasized the clinical importance of *Y. enterocolitica* and probably indicated its presence in Nigeria.

Furthermore, the consumption of pork and dog (reservoir hosts) meat within this environment was also identified to be one of the major causes of the increased rate of isolation due to poor processing and undercooking of the meat products as observed by Ostroff et al. (1994).

The CIN selective medium and the cold enrichment method used in this study probably enhanced the isolation of *Y. enterocolitica* as documented in similar findings (Schiemann, 1979; Pai et al., 1979). *Y. entero-*

colitica biotype 2, 4 and serotype 0:9 were prevalent in this study but different from those seen in Europe (Hoogkamp-Korstanje and Stolk-Engelaer, 1995; Bottone, 1999). The antibiotic susceptibility profiles of *Y. enterocolitica* to ciprofloxacin, floxavid and streptomycin are similar to reports of Okwori et al. (2005), who documented sensitivity of *Y. enterocolitica* strains of animal origin to ciprofloxacin and floxavid. This finding further buttresses the fact that incidence of *Y. enterocolitica* in this part of the world is mostly due to animal faecal contamination.

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