Occurrence and Antimicrobial Susceptibility of *Yersinia enterocolitica* in Diarrhoeic Humans from University of Abuja Teaching Hospital, Gwagwalada, Abuja

*Mailafia S.*, Okoh G. R.¹ and Umunname C.S²

¹Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja

²Department of Biological Sciences, Faculty of Sciences, University of Abuja

**ABSTRACT**

This present study aims at investigating the prevalence of *Yersinia enterocolitica* in stool samples of diarrhoeic patients attending the University of Abuja Teaching Hospital and to determine the antimicrobial susceptibility patterns of the isolates. A total of 200 human stool samples were collected from diarrhoeic patients attending the University of Abuja teaching hospital Gwagwalada. 96 (48%) of the stool samples were from male patients while 104 (52%) were from female patients. The samples were analysed for the presence of *Yersinia enterocolitica* using the conventional Microbial bioassay. Our results showed an overall prevalence rate of 6%. The prevalence rate was higher in females (4%) than in males (2%). However, statistical analysis showed no association between the prevalence of *Yersinia enterocolitica* and sex of host \( P<0.05; (q^2 =3.41); (df= 1) \). All the isolates were tested for susceptibility to commonly used antimicrobial agents. The results of the antimicrobial studies showed that 33.3% (4) of the isolates were susceptible to Ofloxacin, Gentamycin, Nalidixic acid and Nitrofurantoin. 66.7% (8) isolates were resistant to Ofloxacin, Cotrimoxazole, Nalidilic acid, Tetracyclin and Nitrofurantoin. All isolates (100%) showed resistance to Augemetin and Amoxicillin. Our study clearly showed that *Yersinia enterocolitica* is resistant to commonly used antimicrobial agents. However, it is important for tests to be conducted to determine susceptibility before drug usage. Also, serotyping to determine the specific antigenic strains is necessary for effective control of field strains and vaccines possibility is warranted.

**Key words**: Antimicrobial susceptibility, diarrhoeic patients, Prevalence, stool samples, *Yersinia enterocolitica*

*Corresponding email: smailafia@gmail.com
Tel: +234 (0)803 292 2883

This Paper was accepted on 16th November, 2017 and published 24th April, 2018
INTRODUCTION

*Yersinia enterocolitica* is an emerging pathogen belonging to the genus *Yersinia* and consists of both pathogenic and non-pathogenic strains [1]. It is an enteric organism belonging to the family of bacteria the *Enterobacteriaceae* and is one of the most important animal and human pathogen [2] [3]. *Yersinia enterocolitica* is divided into six biovars namely 1A, 1B, 2, 3, 4 and 5 and more than 50 distinct serotypes (O:8, O:3, O:5, 27, O:13a,13b, O:20, O:9, and so forth), with only a few of them being pathogenic[4] [5]. The genus *Yersinia* has eleven sub-species which include: *Y. aldovavm Y. berovieri, Y. frederikenii, Y. intermedia, Y. mollaretti, Y. kristensenii, Y. pestism Y. pseudotuberculosis, Y. rhodeim Y. enterocolitica and Y. ruckei*[6] [5]. *Yersinia enterocolitica, Y. pestis and Y. pseudotuberculosis* are the most widely studied and economically important of all the species [5]. This is because they have been implicated in a host of *Yersinia* related infection as well as diseases involving humans [7].Transmission of this organism is via faecal contaminated foods such as pork [8], vegetables like carrots, tomato, mushroom and even unpasteurized milk [9] [10].

The bacterium was first reported by McIver and Picke, in 1934 [11]. Schleifstein and Coleman provided the first recognized description of five human isolates of *Y. enterocolitica*, in 1939 [12]. *Yersinia enterocolitica* is gradually emerging globally since its first isolation [13] [5] as an enteric pathogen responsible for a wide variety of clinical manifestations such as mesenteric lymph adenitis, diarrhoea, *endocarditis* [14] as well as *acute gastroenteritis* [15] [16]. The European continent has recorded cases of infections with *Y. enterocolitica* in countries such as Germany, France, Finland, Austria, Denmark, Portugal Ireland, Greece, Spain, Scotland, Northern Ireland Sweden and so on [17]. Reports from Africa and Asia further attest to a probable worldwide occurrence of this pathogen [5] [18]. In the United States of American, the first case of *Yersinia enterocolitica* was reported in New York in 1972 [19] and between 2001 and 2008, a total of 47,627 cases of *Y. Enterocolitica* have been reported in globally with an annual statistics of about 4,354-7,540 cases [20].*Yersinia enterocolitica* is a zoonotic pathogen that replicates in the terminal ileum [2] causing gastrointestinal disease in humans, as well as reactive arthritis and erythema *nodosum*[21]. The United Nations International Children Education Fund (UNICEF) has estimated the annual death figure due to *Yersinia* related diarrhoea to be about 194,000; only second to malaria [22].

In Gwagwalada area council, there is a paucity of information on the occurrence of *Yersinia enterocolitica*. Therefore, this study is important as it aims at investigating the prevalence of *Yersinia enterocolitica* in stool samples of diarrhoeic patients attending the University of Abuja Teaching Hospital and to determine to antimicrobial susceptibility patterns of the *Yersinia enterocolitica* isolates.
from the stool samples.

MATERIALS AND METHODS

Study area
This study was carried out in Gwagwalada metropolis, Federal Capital Territory (FCT), Abuja. Gwagwalada is one of the six (6) Area Councils of the Federal Capital territory, Abuja; alongside Abaji, Kuje, Bwari, Kwali and Abuja Municipal Area Council within the Guinea savanna zone. It covers an estimated land mass of 1,043 km² and a population of 157,770, where the University of Abuja teaching hospital is located [23] [24]. Gwagwalada is located on geographical coordinates of 8°56′29″ North, 7°5′31″ East (3D Google Earth).

Collection and preparation of samples

Sample collection
A total of 200 freshly voided human stool samples were collected in sterilized bijou bottles from diarrhoeic patients attending the University of Abuja teaching hospital Gwagwalada between the months of August and September. 96 of these stool samples were collected from male patients while 104 were collected from female patients.

Cold enrichment
About 1-2 gramme (g) of the faecal sample was homogenized in a tube containing 10 ml of phosphate buffered saline (pH 7.2) and homogenised for 30 seconds (s). The homogenized samples were then incubated at 4°C for 10 days, thereafter, sub-cultured unto MacConkey Agar (MCA) and Cefsulodin Irgasan Novobiocin (CIN) Agar. The culture plates were incubated at 28°C for 24 hours (hrs) [18] [25].

Bacterial culture, isolation and identification
Using a flame sterilized wire loop, streaks were made on MCA (Oxoid, UK) from homogenized samples in each sterile bottle, with the procedures being repeated for each sample in different sterile bottles. The cultures were then incubated at 28°C for 24hrs after which distinct colonies with such characteristics as non-lactose fermenting, circular, smooth, convex and an entire edge were isolated. The suspected *Y. enterocolitica* isolates were then sub-cultured unto CIN Agar (Oxoid, UK) at 28°C for 24hrs. The isolates were examined macroscopically and microscopically after incubation for typical *Y. enterocolitica* colonies on CIN agar (deep-red centre surrounded by a transparent border). Suspected colonies were further subjected to motility test by the hanging drop technique both at 25°C and 37°C. Biochemical reaction used to confirm the isolates was urease test [26] [27] [18].

Antimicrobial susceptibility
To determine antimicrobial resistance, a simple media Nutrient agar (NA) plates were prepared following manufacturers advice (Oxoid, UK) by dissolving 28g of the powdered media in 1 litre of distilled water in conical flaks after which the mixture was sterilized at 121°C for 15 minutes using autoclave. After sterilization, the media was allowed to cool to a suitable temperature to allow for pouring and after pouring in sterile
Petri-dishes, the media was then allowed to set. Isolates in the bijou bottles were homogenized in nutrient broth and then inoculated into the nutrient agar plates by a spread plate method using a hockey stick to spread the inoculums after which it was left standing form some minutes. Antibiotic disk of choice was then placed using a sterilized forcep on the nutrient agar plate inoculated with the test organism and then incubated for 24hrs at 28ºC. The sensitivity spectrum of each of the isolates to eight (8) different commonly used antibiotics was determined by standardized single disc diffusion method [28] [29].

The results were interpreted as follows: Presence of visible growth in the negative control but absent around the edges of the antimicrobial disks to about 18mm and above was described as susceptible, presence of visible growth in the negative control but absent around the edges of the antimicrobial disks to between 13-17mm was described as intermediate, while presence of visible growth in the negative control as well as the test sample or clear zones of less than 13mm was described as resistant [28] [30] [29].

Statistical analysis

Statistical Package for the Social Science (SPSS) was used for the data analysis. The prevalence rate was expressed as percentage. Data were also extrapolated as pie chart while Chi-square ($\chi^2$) was used to compare the level of association between the prevalence of Yersinia enterolitica and the sex of the host. P<0.05 was considered significant.

RESULTS

Of the 200 stool samples screened for the presence of Yersinia enterocolitica, 12 (6%) were positive (Figure 1). Table I shows the prevalence of Yersinia enterocolitica in stool samples of adults and children with 0% prevalence in children and 6% prevalence in adults. The prevalence rate was higher in female (4%) than in male (2%). However, statistical analysis showed no association between the prevalence of Yersinia enterocolitica and sex of host [P<0.05; ($\chi^2=3.41$); (df= 1)].

All isolates of Y. enterocolitica were non lactose fermenters, urease positive and are motile at 25°C but non motile at 37°C. The colonies on CIN agar appeared with deep red centre surrounded by a transparent border [31] in contrast to those of other enteric bacteria most of which were pink to colourless in nature [18].

The antimicrobial susceptibility patterns from the analysis carried out from the isolates (see Table II) showed that 33.3% (4) of the isolates were susceptible to Ofloxacin, Gentamycin, Nalidixic acid and Nitrofurantoin, 66.7% (8) isolates were resistant to Ofloxacin, Cotrimoxazole, Nalidilic acid, Tetracyclin and Nitrofurantoin. Total resistance was observed in all the isolates 100% (12) to Augemetin and Amoxicillin.
Table I: Prevalence of *Yersinia enterocolitica* isolates from stool samples based on sex and age.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Sex</th>
<th>Number of samples collected</th>
<th>Prevalence rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults (20 and above)</td>
<td>M</td>
<td>92</td>
<td>4 (2.0%)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>96</td>
<td>8 (4.0%)</td>
</tr>
<tr>
<td>Children (1-12)</td>
<td>M</td>
<td>4</td>
<td>- (0.0%)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8</td>
<td>- (0.0%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>200</td>
<td>12 (6.0%)</td>
</tr>
</tbody>
</table>

*P* < 0.05; (χ² = 3.41); (df = 1). **Key:** % = percentage, M= male, F = female

Table II: Antimicrobial susceptibility of *Yersinia enterocolitica* isolates from stool samples.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Susceptible (%)</th>
<th>Intermediate (%)</th>
<th>Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxa cin (5µg)</td>
<td>33.3</td>
<td>0</td>
<td>66.7</td>
</tr>
<tr>
<td>Gentamicin (10µg)</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>Nalidilic (30µg)</td>
<td>33.3</td>
<td>0</td>
<td>66.7</td>
</tr>
<tr>
<td>Nitrofurantoin (200µg)</td>
<td>33.3</td>
<td>33.3</td>
<td>66.7</td>
</tr>
<tr>
<td>Tetracycllin (25µg)</td>
<td>0</td>
<td>33.3</td>
<td>66.7</td>
</tr>
<tr>
<td>Cotrimoxazole (25µg)</td>
<td>0</td>
<td>0</td>
<td>66.7</td>
</tr>
<tr>
<td>Augmentin (30µg)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Amoxicillin (25µg)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

**Key:** % = percentage; µg = microgram

Figure 2: A pie chart showing the number (%) of positive and negative samples

**Key:** % = Percentage; +ve = positive; -ve = negative
DISCUSSION

The results of this study conducted in Gwagwalada area council revealed the occurrence \textit{Y. enterolitica} in humans. The findings of this study are in conformity with previous reports [32] [33]. The overall prevalence rate of 6% recorded in this study is lower than 15% reported in Jos, Nigeria [18]. This variation could be due to impaired or compromised immunity, dietary, social and sanitary habits [34] [31] [35] [36]. However, this finding is in agreement with other studies which attest to a lower prevalence in Africa and particularly in Nigeria [37] [38] [39]. This study shows a prevalence rate of 6% and none (0%) in adults and children respectively. This is in contrast with the findings of previous researchers. A prevalence rate of 5% was recorded in adults and 7.5% in children in Jos, Nigeria [18]. A prevalence rate of 1.4% of \textit{Yenterocolitica} strains was documented from faecal samples of children in Enugu, Nigeria [38]. A similar study reported prevalence rate of 32.8% among diarrhoeic children in Benin, Nigeria [40]. This difference in the prevalence rate of \textit{Y. enterolitica} in humans may be due to many factors such as poor culinary practices, low level of personal and environmental hygiene [18]. The consumption of poorly processed and undercooked pork and dog (reservoir hosts) meat within this environment was also incriminated to be one of the major causes of the increased rate of prevalence [34]. Furthermore, increased isolation of \textit{Y. enterolitica} has been attributed to cold climatic condition [41]. This may explain the low isolation of \textit{Y. enterolitica} from children in this present study. Gwagwalada Area Council is extremely hot in terms of temperature with a mean daily temperature of 31°C [24]. Thus, the low prevalence recorded in this study may be due to high level of personal and environmental hygiene, good culinary practice, low consumption of poorly processed and undercooked pork and dog meat and the hot weather condition of the study area. A higher prevalence was recorded in adults than children in this current study which contradicts and at the same time conforms to the findings of other researchers [18] [42]. The lower prevalence in children may be attributed to small sample size collected during the course of this study. It was also observed in this study that females had higher prevalence (4%) than males (2%). The high prevalence of \textit{Y. enterolitica} in females could be due to impaired or compromised immunity [31]. This may have far reaching consequences as most female individuals are directly or indirectly involved in food handling and preparation, thus, may aid the spread of the organism to their immediate family members and even the population at large.

The result of the antimicrobial susceptibility showed that 33.3% of the isolates were susceptible to Ofloxacin, Gentamycin, Nalidixic acid and Nitrofurantoin, 66.7% isolates were resistant to Ofloxacin, Cotrimoxazole, Nalidilic acid, Tetracyclin and Nitrofurantoin. All isolates (100%) showed resistance to Augemetin and Amoxicillin. Our study clearly showed that \textit{Yersinia enterolitica} is resistant to commonly used antimicrobial agents. This is agreement with previous
workers [43] [44] who reported high rates of resistance of Y. enterolitica to some antimicrobial agents. The resistance pattern displayed in our study may be associated with indiscriminate use, misuse or overuse of antibiotics which results in the emergence of antibiotic resistance factors leading to increased cost of treatment and control [45].

In conclusion, this study has provided a baseline data for informational dissemination on the occurrence of Y. enterolitica in diarrhoeic patients in Gwagwalada, Abuja. Serotyping is necessary to determine the specific antigenic strains involved in this study area to provide for effective control of outbreaks and possible vaccine development is thus warranted. Y. enterolitica was found to be resistant to commonly used antibiotics. Hence, the phenomenon of antibiotic drug use and misuse in Nigeria should be discouraged as this has greatly undermined its effectiveness and potency, thus giving rise to drug resistance by microorganisms.

REFERENCES


21. Drummond, N., Murphy, B.P., Ringwood, T., Prentice, M.B.,


