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Biotyping and Serological Characterization of *Yersinia enterocolitica* Isolates In Human and Pigs in Selected Farms and Hospital in Shango Community, Minna, Niger State, Nigeria

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ABSTRACT: Biotyping and serotyping are essential medical investigations is used to characterize different bacteria types based on reaction to biochemical tests, while serology is used to checks for the presence of antibodies or other substances in blood sample. Therefore, the objective of this study is the biotyping and serological characterization of *Yersinia enterocolitica* isolates in human and pigs in selected farms and hospitals in Shango Community, Minna, Niger State, Nigeria using standard methods.Slide agglutination technique yielded serotype occurrenceof *Yersinia enterocolitica*as A(3), B(31), C(4) and D(7), while Commercial Latex Agglutination Kit (CLAK) gave serotypesA(0:3), B(0:5), C(0:8) and 1of the 6 D isolates were serotype 0:9.*Yersinia enterocolitica*isolates were identified as biotypes 1A (38), 2(4), 4(3), serotypes 0:3, 0:5, 0:8 and 0:9 while 6(E) were non-typable.The biotyping of 15 *Y. enterocolitica* isolatesfrom the human faecal samples yielded eleven *Y. enterocolitica* isolatesbelonging to biotype 1A, one *Y. enterocolitica* isolates of biotype 2 and three *Y. enterocolitica* isolates of biotype 4. The *Y. enterocolitica* isolatesfrom the pig's faecal samples were found to belong to the following biotypes: twenty-seven *Y. enterocolitica* isolatesof biotype 1A, three *Y. enterocolitica* isolatesbiotype 2.Higher frequency of biotype 1A is an indication of possibility of yersiniosis in the studied areas i.e presence of pathogenic*Y. enterocolitica* isolates.The serological typing of the *Y. enterocolitica* isolates from the faecal samples of the diarrhoeic patients were found to contained three 0:3 serotype, ten 0:5 serotype, one 0:8 serotype and one 0:9 serotype. The serological typing of the *Y. enterocolitica* isolates from the faecal samples of the pigs were found to contained twenty-one 0:5 serotype, three 0:8 serotype and six un-typable. The serotype 0:3 found in this study from the human isolates is an indication of presence of acute human yersiniosis in the studied area. It is suggested that antisera specific for each species of *Y.enterocolitica* be used in sero-analysis.

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Y. enterocolitica is a zoonotic pathogen that has been identified as a major cause of bacterial gastrointestinal disease in many developed and developing countries around the world (Rahman et al., 2011) and is the third most common cause of food-borne gastrointestinal disease in Europe (Fredriksson-Ahomaa et al.,2007b;Chlebicz and ´Sli˙zewska, 2018). *Y. enterocolitica* is a well-known food- borne pathogen (Fredriksson-Ahomaa and Korkeala,2003; Zadernowska et al.,2013) and is ubiquitous in domestic and wild animals (Kot *et al*.,2005; Liang et al., 2015; Arrausi-Subiza et al., 2016; Cilia et al., 2021; Modesto et al., 2021; Nasser et al., 2023); and free – livin birds (Odyniec *et al*., 2020). Pigs are considered to be the main reservoir of pathogenic strains for humans, but many species of farm, free-living, and companion animals are susceptible to infection (Laukkanen-Ninios et al., 2014; ECDC, 2020). In most affected animals, the infection is asymptomatic, and the pathogen is abundantly

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excreted with feces and contaminates the environment.Pigs are considered to be the major source of *Y. enterocolitica* human infections (Laukkanen-Ninios et al.,2014; Syczyłoet al., 2018; Terentjevaet al., 2022; Angelovska, et al., 2023).Of the various food products surveyed in countries with systems to monitor *Y. enterocolitica* infections, meat and meat products are widely found as important vehicles for this pathogen and are implicated in several high-profile outbreaks of yersiniosis (Sakai et al., 2005; Zdolec and Kiš 2021). *Y. enterocolitica* is highly resistant to adverse conditions, and it can easily adapt to the environment outside the host organism. The pathogen is able to survive within a pH range of 4.2 to 9 (Vasala et al., 2014) and in water with up to 7% salinity (Espenhain et al., 2019; Strydom et al., 2019). Above all, *Y. enterocolitica* has unusual psychotropic properties. It can survive within a wide range of temperatures, especially at low temperatures, and it can compete with most foodborne pathogens thathave a preference for higher temperatures. *Y. enterocolitica* is able to grow at 28–29^{0}C and can survive freezing conditions (Inns et al., 2018; Hunter et al., 2019). *Y. enterocolitica* pathogenicity is strongly related to the bioserotypes of this bacterium (Rahman *et al*., 2011).The genome size of *Y. enterocolitica*biotypes ranges from 4.5Mb to 4.9Mb. The genome size of all low pathogenic biotypes is 4.5Mb and 4.6Mb for the high-pathogenic biotype while the genome size of the non-pathogenic biotype is around 4.9Mb. The low pathogenic biotype 4 genome has the lowest number of coding sequences (CDSs) while the non-pathogenic biotype 1A include the largest number of CDSs (Reuter et al., 2012). It has been speculated that the serotype O:5, 27, which dominates in the animal population but is a quite uncommon cause of human infections in Great Britain, is either poorly transmissible or relatively nonpathogenic to humans (McNally et al., 2004). Serotypes O:3, O:9 and O:5,27 were found to be the most dominant in Europe (Chart and Cheasty, 2006) while serotype O:8 is the most predominant in North America (Schaake et al., 2013).However, there no literature on biotyping and serotyping studies in Nigeria. Hence, the objective of this study is the biotyping and serological characterization of *Yersinia enterocolitica* isolates in human and pigs in selected farms and hospitals in Shango Community, Minna, Niger State, Nigeria.

MATERIALS AND METHODS

Study area: The study was carried out in Shango community in Minna, Niger State, Nigeria between Latitudes 9º336'81'N and Longitudes 6º355.44'E (Fig.1). Stool samples from both apparently healthy and diarrhoeic patients and healthy pigs were collected from Hospitals and Piggery respectively and assessed for their *Y. enterocolitica* species contents from this community.

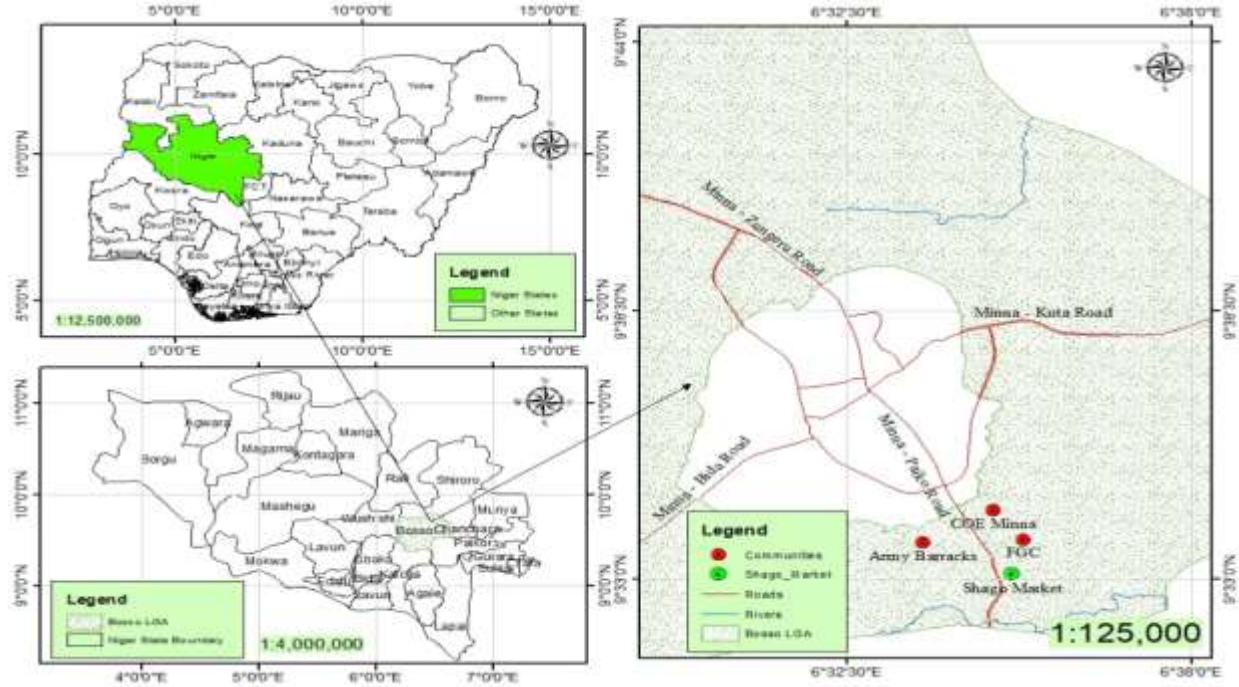

Fig. 1: Map of Study Area (Shango community, Minna)
Source: Adapted from Minna Street Map, 2014

Ethical approval: Ethical approval was obtained from the Ministry of Health, Niger state, before the commencement of sample collection.

Study design: The research, a cross sectional study, and the sample were collected from both male and female patients. Clinical data for each diarrhoeic patient were collected, such as the age, gender, educational and occupational status, using structured questionnaire, to identify the possible socio-demographic factors that assessed a patient's hospital and community risk for Yersiniae acquisition.

Study participants: These comprised of patients with clinically diagnosed cases of diarrhoea. Informed consent was obtained from the patients concerned.

*Sample Collection from Hospitals (Human source):*A total number of two hundred freshly voided faecal samples from apparently healthy individuals and diarrhoeic patients were collected into sterile sample bottles from Hospitals (Primary Health care Centre, Shango, Minna and Babangida Specialist Hospital Minna, Niger State). Sixty faecal samples from the paediatric wards and 140 faecal samples from the Adults males/females wards.

Sample Collection from Animal Farms (pigs):A total number of one hundred and fifty faecal samples from healthy pigs (with no history or signs of illness) were collected into sterile sample bottles from the farms. Fifty samples each from designated farms (A, B and C). All faecal samples collected in sterile sample bottles in this study, were transported immediately to the laboratory and processed within two to three hours of collection/ defaecation for microbial analysis.

Isolation of Yersinia enterocolitica: A loop full of fresh faecal samples from pigs and diarrhoeic human patients were prepared respectively as a 10% suspension in 0.067M phosphate buffer (PBS, pH 7.6) in a sterile labelled test tubes and incubated at 4°C for 3-7 days according to methods described previously (FDA/CFSAN, 2001; Okwori et al., 2005; Van Damme et al.,2013b). The enrichment cultures were then streaked respectively and aseptically onto MacConkey agar and Deoxycholate agar plates and incubated at 37°C for 48 hours according to Standard methods (Weissfeld and Sonnenwirth, 1980). All the non-lactose fermenting colonies from Deoxycholate and MacConkey agar plates were sub-cultured onto freshly prepared MacConkey agar for purity and further identification.

Direct plating of the faecal samples were equally done onto MacConkey agar and Deoxycholate agar plates and incubated at 37^0C for 24-48 hours. After an initial examination, the colonies were purified by sub-culturing the colonies onto another MacConkey agar and Deoxycholate agar plates, returned to the incubator for another 24-48 hours. After 48 hours of incubation, morphological characteristics of the isolates were recorded. Every culture and subculture was properly labelled. The pure non-lactose fermenting colonies (small 1-2mm diameter), flat, colourless, or pale pink colonies from MacConkey agar plates were sub-cultured onto nutrient agar slants and stored at 4°C. Throughout this study stock cultures were maintained on nutrient agar slants stored at 4°C with periodic subculture to maintain their viability (Barrow and Feltman, 1993).

Identification of Isolates: The essential preliminary screening tests include: Cultural and Morphological Characteristics: The cultural characteristics of the isolates were confirmed on MacConkey agar. Smears were made from typical colonies i.e small non-lactose fermenting colonies by emulsifying the organism in a small drop of normal saline on the glass slide to give an even suspension. This was air-dried and the preparation was fixed by passing through a Bunsen flame and was Gram stained. Those colonies that stained Gram- negative and appeared coccobacillary were further tested by examining a Giemsa stained smear for coccobacillary showing bipolar staining. Biochemical characteristics, (viz : catalase production, urease production, Nitrate reduction, Citrate utilization, Oxidase production, Phenylalanine deaminase, Ornithine decarboxylase, Indole production, Hydrogen sulphide production, Esculin hydrolysis, Pyrazinamidase, and Beta-D-glucosidase), Motility and test for metabolism of carbohydrates this includes: Monosaccharides (Glucose, xylose, and sorbose), Disaccharides (Sucrose, Lactose and Trehalose), Polyhydric alcohol (inositol) and Glycoside (Salicin) were carried out as described by Barrow and Feltman (1993), Wang et al. (2009) and Liang et al. (2012).

Biotyping and Serological Procedure: Forty-five (45) pure isolates of *Y. enterocolitica* organism (15 from diarrhoeic human and 30 from pigs), isolated from pig farms and Hospitals in Shango, Minna, Niger State were used for biotyping and serological analysis.
Biotyping Procedure: Biotyping was performed according to thescheme described by Wauters et al. (1987) and Petersen et al.(2015) consisted of the following biochemical reactions: Pyrazinamidase activity, Ornithine decarboxylase, Indole production, Beta-D-glucosidase test, Esculin Hydrolysis, Lipase activity, Salicin acidification, Inositol acidification,

Trehalose acidification, Xylose acidification, Sorbose acidification and Nitrate reduction.

Serological Analysis: The *Y. enterocolitica* isolates from the diarrhoeic human (15) and pigs (30), sterile TSB, 20mls needle and syringes, sterile test tubes and pippetes, rabbits and commercial *Y. enterocolitica* serotyping kits(ITEST-plus s.r.o Czecks Republic).

Preparation of Antisera: Three isolates each of *Yersinia enterocolitica* from the diarrhoeic human (15) and pigs (30) were selected randomly for antigen preparation as follows:

Broth Preparation: Fresh TSB were inoculated respectively with the three isolates each of *Yersinia enterocolitica* from diarrhoeic human and the pigs into separate labeled test tubes and incubated for 18hours. The cultures were heated at 100^0C for 1 hour for the preparation of the *Yersinia enterocolitica* antigen. Six apparently healthy rabbits were inoculated each with the *Yersinia enterocolitica* antigen by injecting into the ear vein 0.5ml of the antigenic culture, then with 1ml on the third day, then 2mls on the 5th day, 4mls on the 8th day, 8mls on the 16th day, 16mls on the 20th day, and 1 week later 16mls was again inoculated to ear vein by the methods previously described by (Sofroniew et al., 1978; Mastrodonatoet al., 2018). The inoculated rabbits were left for one week for observation. Then they were sacrificed by heart puncture with 20mls of syringe and 25mls gauge of needles to collect the blood and centrifuged for determination of antibody titre. The sera obtained were carefully kept for agglutination reactions with the *Yersinia enterocolitica* isolates from the diarrhoeic human and pigs

Agglutination Reaction: The serum obtained from each inoculated rabbits was reacted with each of the *Yersinia enterocolitica* from diarrhoeic human and pigs on a cleaned slide and observed for agglutination for preliminary grouping.

Determination of Titre: To determine the titre of each antiserum;a pure culture of each of the *Y.enterocolitica* used for inoculating the rabbit was inoculated into 10 mls of TSB. This was incubated at 37^0C for 48hours. Ten test tubes (sterile) were then arranged in a rack.

The first tube contained 9ml of TSB; the others contained 5ml each. One mililitre of *Y. enterocolitica* antisera was inoculated into the 1st tube. Serial dilution was carried out after thorough mixing in the 1st tube. 5ml was transferred to the 2nd tube; the procedure was done to the last tube where 5ml was discarded. The culture was incubated at 37^0C for 24hours and the titre was read. With the antisera produced the *Yersinia enterocolitica* isolates were grouped into three groups each for the human and pig's isolates. The procedure was repeated for the two other antiserums.

Serotyping: Serotyping of the isolates were carried out using *Y. enterocolitica* antisera commercial kit for0:3, 0:5, 0:8, and O:9 (ITEST-plus s.r.o Czecks Republic). The inoculums of the isolates were prepared and a drop each was placed on a clean slide and a drop of the antisera was added and gently mixed. Agglutinations were noted and recorded for all the *Y. enterocolitica* isolates in the study as described by Sivonen et al. (2009).

RESULTS AND DISCUSSION

A total of Fifteen (15) *Y. enterocolitica* were isolated from faecal sample of human diarrhoeic faecal samples which account for 7.5% of the sampled size and a total of thirty (30) faecal samples of the pigs had the presence of *Y. enterocolitica* infection which account for 20.0% of the sample size of pigs faecal sample. There is significant difference statistically between the isolation made from human stool samples and pigs faecal samples ($P < 0.05$) (Table 1 and Table 2).

The biotyping of 15 *Y. enterocolitica* isolates from the human faecal samples yielded eleven *Y. enterocolitica* isolates belonging to biotype 1A, one *Y. enterocolitica* isolates of biotype 2 and three *Y. enterocolitica* isolates of biotype 4 while the *Y. enterocolitica* isolates from the pig's faecal samples were found to belong to the following biotypes: twenty-seven *Y. enterocolitica* isolates of biotype 1A, three *Y. enterocolitica* isolates biotype 2 (Table 3 and Table 4).

The serological typing of the *Y. enterocolitica* isolates from the faecal samples of the diarrhoeic patients were found to contained three O:3 serotype, ten O:5 serotype, one O:8 serotype and one O:9 serotype.

The serological typing of the *Y. enterocolitica* isolates from the faecal samples of the pigs were found to contained twenty-one O:5 serotype, three O:8 serotype and six un-typable (Table 5).

Table 1:*Y. enterocolitica* isolates from pigs and human diarrhoeic faecal samples.

Sources	Number of samples	Number of isolates	% Occurrence
Humans	200	15	7.5
Pigs	150	30	20.0

$\chi^2 = 11.95 > CV = 3.841; df = 1; p<0.05$

Table 2: Biochemical characteristics of *Y. enterocolitica* isolates from pigs and human diarrhoeic faecal samples

Reactions	Results
Cultural properties	Non-lactose fermenting
On MacConkey Agar	Colonies (small 1-2 diameter) flat,colourless or palepink
Gram staining	Gram negative rod
Motility at 25^0C	Actively motile
Motility at 37^0C	Non motile
Fermentation of sugar	Positive
Glucose (Acid production)	
Sucrose	Positive
Trehalose	Positive
Salicin	Negative
Lactose	Negative
Inositol	Positive
Xylose	Positive
Sorbose	Positive
Catalase test	Positive
Urease production	Positive
Nitrate reduction	Positive
Citrate utilization	Negative
Oxidase test	Negative
Phenylalanine deaminase	Negative
Oxidase test	Negative
Ornithine decarboxylase	Positive
Indole production	Positive

Biotyping and serotyping have historically been used to differentiate between isolates beyond the species level. *Y. enterocolitica* isolates that have the same biotype or serotype are more likely to be related to each other than if they had different biotypes or serotypes. Therefore, these subtyping methods are a useful starting point for public health surveillance purposes. Incidence of yersiniosis that is caused by a particular biotype and serotype at levels higher than expected may indicate an outbreak and will require further investigation. To assist public health teams with their outbreak investigations it is essential that subtyping provides an appropriate level of discrimination to link epidemiologically associated cases. Enteropathogenic Yersinia circulate in the pig reservoir, in West Africa reports of human yersiniosis are rare. This study was conducted to determine whether pathogenic Yersinia are circulating in pig farms and are responsible for human infections in the Central Northern Nigeria region (Shango, Minna, Niger State) (Table 1 and Table 2). About 54 serum groups according to antigen O have been described but O:3, O:5, O:8 and O:9 are usually prevalent in human pathology.

These are entero-invasive and/or destructive strains (infective dose 10^8-10^9 organisms/ml) which in a typical way invade the colon and terminal ileum multiplying within the mucous cells, the mastocytoma and the Peyerian glandsor patches (McNally et al., 2006; Bottone, 2015). Besides, they can elaborate enterotoxins responsible for food poisoning (FoodNet, 2013).

Table3: Differentiation of *Yersinia enterocolitica* isolates from diarrhoeic faecal samples of human and pigs in the study into biogroups

Tests	Bio-group reactions					
	1A	1B	2	3	4	5
Indole production	+	+	+	-	-	-
Esculin hydrolysis	+	-	-	-	-	-
Pyrazinamidase	+	-	-	-	-	-
βD-Glucosidase	+	-	-	-	-	-
Lipase activity	+	+	-	-	-	-
Ornithine decarboxylase	+	+	+	+	+	-
Nitrate reduction	+	+	+	+	+	-
Acid from:						
Inositol	+	+	+	+	+	+
Salicin	+	_	-	-	-	-
Sorbose	+	+	+	+	+	-
Trehalose	+	+	+	+	+	-
Xylose	+	+	+	+	-	+

Table4: The Biotyping patterns of *Y. enterocolitica* isolates from diarrhoeic faecal samples of human and pigsfrom the study. N=15 for the human isolates, N=30 for the pigs isolates.

Biotypes/ Isolates	1A	1B	2	3	4	5
Human isolates (*Y.enterocolitica)*	11	-	01	-	03	-
Pig isolates (*Y.enterocolitica)*	27	-	03	-	-	-

Table5: The serological typing of *Y. enterocolitica* isolates from diarrhoeic faecal samples of human and pigs from the study. *N=15 for the human isolates, N=30 for the pigs isolates.*

Antisera/ Isolates	O:3	O:5	O:8	O:9	Un-typable
Human Isolates (*Y.enterocolitica)*	03	10	01	01	-
Pigs Isolates (*Y.enterocolitica*)	-	21	03	-	06

In this investigation eleven (77%) *Y. enterocolitica* isolatesfrom the human faecal samples were biotype 1A, one (6.7%) *Y. enterocolitica* isolates belong to biotype 2 and three (16.7%) *Y. enterocolitica* isolates were biotype 4.The *Y. enterocolitica* isolatesfrom the pig's faecal samples were found to contained the following biotypes: twenty-seven (90.0%) *Y. enterocolitica strains* of biotype 1A, three (10.0%) *Y. enterocolitica strains* of biotype 2.A total number of thirty-eight (85.6%) *Y. enterocolitica* isolates from the study were biotype 1A; four were biotype 2 and three were biotype 4(Table 3 and Table 4). The biovar 1A strains of *Y. enterocolitica* are isolated worldwide from diarrhoeic human subjects and environmental sources (Bottone, 1997, Bottone, 2015). These have been regarded as avirulent in the past because they lack the genotypic and phenotypic markers of virulence, which are generally used to assess the pathogenicity of *Y. enterocolitica* (Tennant *et al.*, 2003). However, some studies have shown biovar 1A strains to be the etiological agents of gastroenteritis in children(Abdel-Haq et al., 2000; El Qouqa et al., 2011; Riahi et al.,2021**).***Y. enterocolitica* biotype 1A strains

are able to synthesize variants of thermostable YST enterotoxin and play a key role in the pathogenesis of yersiniosis because enterotoxins provoke diarrhoea, which is the main cause of mortality in yersiniosis (Ramamurthy et al., 1997; Sakaiet al., 2005; Huovinen et al., 2010; Bancerz-Kisiel et al., 2012; Morka et al., 2021). YST enterotoxins produced by *Y. enterocolitica* stimulate the activation of guanylate cyclase, which increases the concentration of cyclic guanosine monophosphate (cGMP) in epithelial cells and leads to fluid accumulation in the intestine (Bancerz-Kisiel et al., 2014). Biotype 1A strains of *Y. enterocolitica*have also been confirmed to produce *Y. enterocolitica* pore-forming toxins, YaxA and YaxB. These toxins form pores in the cell membrane of host target cells and cause osmotic lysis, which is of particular importance in systemic infections (Wagneret al., 2013; Bräuninget al., 2018; Platt-Samoraj, 2022).In Plateau State of Nigeria, *Y. enterocolitica* bioserotype 2/O9 was the only isolated pathogenic in human samples (Okwori et al., 2007). The finding in this study is in contrast with several earlier reports which documented lower numbers and percentages of biotype 1A. In Switzerland, 811 fecal samples from asymptomatic humans were studied for the presence of *Y. enterocolitica*. Nine (1.1%) of the 811 samples were positive for *Y. enterocolitica* 1A (Stephan et al., 2013). In Canada, Caprioli et al. (1978) and Marks et al. (1980) reported 13 biotype 1A from 256 human isolates of *Y. enterocolitica* associated with gastroenteritis, 36 biotype 1A from 157 strains of human origin and 12 biotype 1A from 181 strains of *Y.enterocolitica*of gastroenteritis respectively. In the USA, Kay et al. (1983) reported 36 biotype 1A strains of *Y. enterocolitica* from 100 clinical isolates submitted to the US centre of disease control. Approximately 40% of 277 clinical isolates o*f Y. enterocolitica* were biotype 1A from the report of Bissett et al. (1990). Phamet al. (1991) reported 11 cases of *Y.enterocolitica* enteritis in Australia twoof which were found to be biotype 1A and 24 strains from 100 clinical strains were biotype 1A. Onyemelukwe, (1993) reported three biotype 1A strains from the nine *Y. enterocolitica* obtained from the stool samples of diarrhoeic patients in Enugu, Nigeria. Bioserotype 4/O:3 of *Y. enterocolitica* is the major isolated one from humans globally (Fredriksson-Ahoomaet al., 2007) and was isolated in some European countries, including Denmark, Italy, Belgium, Spain, Finland, and Sweden (Sihvonen et al., 2009; Rahman et al.,2011). The finding of this study however correlates with several earlier reports which stated clearly that biotype 1A were the most prevalent from the environmental and clinical sources. In the USA, 72 out of 77 strains of *Y. enterocolitica* from patients with gastroenteritis were documented to be biotype 1A (Greenwood, 1993**).** Noble et al. (1987) also documented 90% of 125 *Y. enterocolitica* strains isolated to be biotype 1A. Forty-nine biotype 1A of *Y. enterocolitica* were obtained from 120 human strains from disease (Shayeganiet al., 1981).Biotype 1A are increasingly being associated with diarrhoea (Morris Jr.et al., 1991; Morka et al., 2021), and all biotype 1A serotypes that causes human infections has been found to be present in oral cavity of pigs indicting pigs as source of infection to human (Ostroff et al., 1994; ECDC, 2020). Another study conducted in Henan province, Chinabetween 2005 and 2011 is in harmony to the present study, as the dominant epidemic serotypes was O:5 , with biotype 1A being the dominant biotype accounting for 84.7% of *Y. enterocolitica*isolates (Mu et al., 2013).Alsobiotype 1A strains are also significantly recovered from Australia, India, and the United States (Pham et al., 1991; Bottone, 1997; Sharma et al., 2004).In general, acute infection with biotype 1A yersiniae tends to mimic infection with pYV-bearing *Y. enterocolitica* in terms of clinical manifestations and severity. A study by Burnens et al. (1996) in Switzerland demonstrated that the duration and severity of infection associated with biotype 1A yersiniae was indistinguishable from that due to classical virulent biotypes. Similar observations have been made in patients from England, Chile and Spain (Morris Jr.et al., 1991; Cimolai et al., 1994; Lobatoet al., 1998**).** In contrast to pYV-bearing *Y. enterocolitica*, however, biotype 1A strains seldom give rise to extraintestinal infections or autoimmune sequelae. Nevertheless, biotype 1A strains have been isolated from abdominal exudates, wounds, sputum, urine, a labial ulcer and the gall bladder (Bottone, 2015), and have been linked to cases of reactive arthritis (Bottone, 1997; Imoto et al.,2012; Vasala et al., 2014; Tuompo et al., 2017). Presumably because they are susceptible to complement- mediated lysis, biotype 1A yersiniae rarely cause septicaemia and have only once been associated with a human death (Butler,1990; Bottone, 2015).Patients who are convalescing from biotype 1A-induced gastroenteritis generally display low or undetectable titres of circulating antibodies to these bacteria (Buttet al.,1991). This has resulted in these strains being overlooked as aetiological agents, because pYV-bearing *Y. enterocolitica*, in particular serotype O:3, frequently evoke serum agglutinating antibody titres of greater than 1 in 200. However, low titres of antibody have also been found in patients with proven infection due to pYV-bearing *Y. enterocolitica*, including serotypes O:5,27, O:1,2,3 and O:21 (Buttet al.,1991), indicating that antibody titre does not necessarily correlate with the virulence of the infecting strain. The pathogenic characteristics of biotype 1A strains of clinical origin have now been found on the

basis of greater capacity to (1) penetrate cultured epithelial cells, (2) survive within macrophages, (3) exocytose from epithelial cells and macrophages and (4) colonise the intestinal tract of orally inoculated mice for prolonged periods provide a variety of convenient models in which to assay the pathogenicity of these bacteria (Grassl et al., 2003; Tennant et al.,2003; McNally et al., 2006).Various studies show that some biovar 1A *Y. enterocolitica* strains, and especially those of clinical and animal origin, invade and survive in cultured epithelial cells better than strains isolated from the environment (Singh and Virdi, 2005; Fredriksson-Ahomaa et al.,2010; Zadernowska et al.,2013; Syczyłoet al., 2018). Such discrepant observations may be due in part to the fact that biovar 1A strains encompass a plethora of serotypes and non-agglutinable (NAG) strains, and thus comprise a highly variable group of strains (Tennant, et al., 2003).On the contrary, the prevalence of strains belonging to biotypes 2 and 4 is much lower, despite several studies reporting sporadic cases from the slaughter of pigs, cows, sheep, goats, monkeys, and wild rodents with potential transmission to humans (Hayashidani et al.,1995; Fearnley et al., 2005).In this study, the serological typing of the *Y. enterocolitica* isolates from the faecal samples of the diarrhoeic patients was found to contain three serotype O:3, ten serotype O:5, one serotype O:8 and one serotype O:9. While the serological typing of the *Y. enterocolitica* isolates from the faecal samples of the pigs were found to contain twenty-one 0:5 serotype, three O:8 serotype and 06 *Y. enterocolitica* isolates were un-typable in this study i. e the serological groups are not included in the antisera employed (Table 5). In summary, thirty-one *Y. enterocolitica* isolates were serotype O:5, three were serotype O:3, four were O:8 serotype and one were serotype O:9. The result of the study differs largely from several earlier studies recorded. Serotype O:3 has been reported as predominant in human and pig population followed by serotype O:5 and 0:9 in the USA and Norway (McNally et al., 2004; Bhaduri and Wesley, 2006). This is attributed to the fact that in *Y. enterocolitica*, geographical origin of the isolates, ecological niches from which they are isolated and their pathogenic significance are closely correlated to certain biovar/serovar combinations (Bottone, 1999). The serotype 0:3 found in this study from the human isolates now confirm the presence in Central Northern Nigeria. From documentation serotypesO:3,O:5, O:6, O:8 and O:9 have earlier been isolated from human and animal sources and documented by previous workers in the Southern parts of Nigeria and Africa (Agbonlahor et al.,1981;Agbonlahor et al., 1983;Onyemelukwe, 1993; Gasco et al., 2000; Omoigberale and Abiodun, 2002;Okwoli et al., 2007; Bublitz et al., 2014; Saraka et al., 2017). The yersinial nuance in the extent of gastrointestinal tract pathologic findings centers largely about the serogroup of the invading strain, with serogroup O:8 producing the more catastrophic events, including extensive ulceration of the gastrointestinal tract and death (Hayashidani et al., 1995; Ackers et al., 2000; Movafagha et al., 2021); whereas serogroups O:3 (Lee et al., 1990; Espenhain et al., 2019) and O:9 are less destructive in the gastrointestinal tract (Batzilla et al., 2011a). This study revealed that pathogenic *Y. enterocolitica* are harboured by pigs since different serotypes common with human infections can be isolated from them. They can therefore be transferred to the humans.

Conclusions: This study revealed varied serotyping and biotyping patterns in humans and pigs isolates and strong possibility of yersiniosis in the studied areas. The paucity of reports of yersiniosis in West Africa is most likely attributable to a lack of active detection of the microorganism. The identification of hypermutator strains in pigs and humans is of concern as these can rapidly acquire selective advantages that may increase their pathogenicity or resistance to commonly used treatments. Therefore it is suggested that Government should set up National Yersinial Surveillance Laboratory Centre to monitor Yersinial infections as recorded in this study.

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