Profiles Of Yersinia Enterocolitica Isolated From Apparently Healthy Pigs In Jos, Nigeria

A Okwori, S Agina, A Olabode, A Ochapa, A Turay, L Lombin

Citation

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Abstract

A total of three hundred and twenty pig samples were collected from Jos and its environs. The samples comprised of tonsils (160) and faeces (160) from five different study areas. Identification of isolates was based on cultural method by the use of cold enrichment and direct plating on differential and selective culture media. Fifty six (56) strains of Yersinia enterocolitica were isolated. They included thirty two (32) from tonsils and twenty four (24) from faeces. Isolates were characterized biochemically with API 20E. They were further biotyped and serotyped. The results showed prevalence rates of 15% and 20% of isolates from faecal and tonsil samples respectively. Biotype 2 and serotype 0:9 predominated. All isolates were susceptible to ciprofloxacin, tarivid and gentamycin. It is obvious from our findings that pigs has an important role as a source of infection. This adds to the growing evidence of pathogenic Y. enterocolitica in Jos, Plateau State, Nigeria.

INTRODUCTION

Yersinia enterocolitica is a common pathogen for both human and animals, the predominant clinical feature is abdominal pain and diarrhea with or without fever [1]. The emergency of Yersinia enterocolitica 0:3 and 0:9 in Europe and Japan in the 1970s and in North America by the end of the 1980s has been characterised as an example of a global pandemic [2]. The number of infection due to Yersinia enterocolitica in humans has increased considerably over the past years [3]. These infections are often linked to pork consumption [4].

There is a strong indirect evidence that swine constitute an important reservoir for human infection with Yersinia enterocolitica [5,6,7,8].

The pig is the only food animal which regularly harbours pathogenic Yersinia enterocolitica [9]. Epidemiological surveys have shown that Yersinia enterocolitica in healthy carriers do exist and certainly may represent an important factor in the spread of this infection. Yersinia enterocolitica has been isolated from a variety of environmental sources but pigs are recognised to be the main reservoir of the different Yersinia enterocolitica serotypes (0:3, 0:9, 0:5, and 0:8) which are also known pathogens prevalent in human infections [3,10] The apparently low incidence of the infection in Islamic countries where consumption of pork is restricted buttresses the fact that pork is the main sources of human infections.

In Nigeria, [11] and [12] reported the oocurrence of Yersinia enterocolitica in pigs in Zaria and Jos respectively. Gastroentritis and diarrhoea associated with Yersinia enterocolitica have been similarly documented by [13] and [14].

This study was aimed at establishing the frequency and profiles of pathogenic Yersinia enterocolitica among apparently healthy pigs in selected herds in Jos and its environ.

MATERIALS AND METHODS

Three pig herds (Kaduna Vom, Kuru, and Vwang) located in Jos South Local Government Area and one large weekly pig market in Kafanchan, Kaduna State (neighbouring Local Government Area to Jos) were selected for this study (Fig.1). A total of 320 tonsil and faecal samples were screened for the presence of Yersinia enterocolitica.

COLD ENRICHMENT

1g of faeces and tonsil swab samples were aseptically transferred into 10 ml of phoshate buffered saline (pH 7.2) (Life Technologies Ltd, Paisely, Scotland), vortexed and homogenized for about 30 s and incubated at 4°C for three weeks and subsequently subcultured into Deoxycholate Citrate Agar (DCA), MacConkey Agar (MCA) and Cefsulodin Irgasan Novobiocin Agar (CIN). The culture

plates were incubated at 30°C for between 18 - 24 h [15] .

BACTERIAL ISOLATION AND IDENTIFICATION

Culture plates (DCA (Oxoid, UK), MCA (Fluka, Sigma Aldrich

Chemie, GmBH, Germany), CIN (Oxoid, UK) were examined. Suspected colonies were further subjected to motility test by hanging drop technique both at 25 and 37°C. Isolates that were motile at 25 ° C but non motile at 37°C were selected. Biochemical test (API 20E, Biomereux, France) including urease activity were used for the bacterial identification [16] .

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 [17] 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 [17] .

ANTIMICROBIAL SUSCEPTIBILITY

The sensitivity spectrum of each of the isolates to eight different antibiotics was determined by standardized diffusion method [18,19] . The antimicrobial agents used were ciprofloxacin (10µg/ml), tarivid (10µg/ml), gentamycin (10µg/ml), streptomycin (20µg/ml), amoxicillin (10µg/ml), ampicillin (10µg/ml), and erythromycin (10µg/ml) (Abtek biological Ltd, Liverpool, UK). The diameters of the zones of inhibition around each antibiotic disc were measured in millimetres.

DATA MANAGEMENT AND ANALYSIS

Chi-square test with Yates continuity correction was used to test if there was any difference in the prevalence of Yersinia enterocolitica 0:9 between faeces and tonsil samples.

RESULTS

Samples from the three pig farms visited in Jos and the weekly pig market in Kafanchan, Kaduna State were all positive for Yersinia enterocolitica. A total of 56 (17.5%) Yersinia enterocolitica strains (Table 1) were isolated from 320 samples of tonsils and faeces. Kafanchan study area gave a high prevalence rate of 7.8%. Of the 160 tonsil samples screened from all study areas 32 (20%) were positive for Yersinia enterocolitica (Table 2) while 24 (15%) of faecal samples were positive for Yersinia enterocolitica (Table 3).

All isolates belonged to predominant human and animal serotypes in Nigeria [14,20] , 18 and 14 Yersinia enterocolitica strains were biotyped 1 and 4 respectively among the tonsil samples while 8 and 16 Yersinia enterocolitica strains were biotyped 1 and 4 among the faecal samples

(Table 4). All strains of Yersinia enterocolitica were susceptible to ciprofloxacin, tarivid, gentamycin and but resistant to amoxicillin, ampicillin, and erythromycin (Table 5). No significant differences were observed among the samples analysed (P<0.5).

Figure 1

Table 1: Percentage distribution of from different study areas

Study Area	Number sampled	Number positive	Percentage (%)
Kafanchan	80	25	7.8
Vom	80	18	5.6
Kuru	80	08	2.5
Vwang	80	05	1.6
TOTAL	320	56	17.5

Figure 2

Table 2: Percentage prevalence of in Tonsil samples

Study Area	Number sampled	Number positive	Percentage (%)
Kafanchan	40	15	9.4
Vom	40	09	5.6
Kuru	40	05	3.1
Vwang	40	03	1.8
TOTAL	160	32	20.0

Figure 3

Table 3: Percentage prevalence of in faecal samples

Study Area	Number sampled	Number positive	Percentage (%)
Kafanchan	40	10	6.3
Vom	40	09	5.6
Kuru	40	03	1.9
Vwang	40	02	1.3
TOTAL	160	24	15.0

Figure 4

Table 4: Phenotypic profiles of pathogenic

Sample	Number of strain	Biotype	Serotype
Tonsils	32	18* (1)[a] 14 (4)[a]	32* (0:9)[b]
Faeces	24	8 (1) 16 (4)	24 (0:9)

* = Number of strains
a = Biotypes
b = Serotype

Figure 5

Table 5: Antimicrobial Susceptibility Pattern Of Strains

Antimicrobial agent	Disc potency (µ/ml)	Number sensitive	Zone of inhibition (mm)	Number resistant
Ciprofloxacin	10	7	27	0
Tarivid	10	7	25	0
Gentamycin	10	7	20	0
Streptomycin	20	7	20	0
Amoxicillin	10	0	0	7
Ampicillin	10	0	0	7
Erythromycin	10	0	0	7

Figure 6

Figure 1: Species of pigs investigated

DISCUSSION

Our findings are further proof that the pig population in the study areas are reservoir of human infections as serotypes and biovars isolated in these herds have been found to be responsible for most epizootics diseases as documented in a similar study by [21] . This is perhaps the only biogroups present among swines in this part of the world. The prevalence rate (17.5%) found in this study are consistent with the rates found by [22] in Denmark and Norway [23] . These findings are also similar to reports in other parts of the world particularly in India [24] and Finland [25] where Yersinia enterocolitica was frequently isolated from intestinal contents of slaughtered pigs and rectal swabs of apparently healthy pigs. The occurrence of Yersinia enterocolitica in pigs investigated in this study seems to be a global problem [26,27] . Unlike other countries where Yersinia enterocolitica serotype 0:3 is frequently encountered in animals and human [26,28] our studies has identified serotype 0:9 as the most prevalence strain in this part of the world [14] .

Although this study can not give complete information about the true prevalence of Yersinia enterocolitica 0:9 in the whole of Nigeria but it can be considered to represent the figures in the middle belt of Nigeria with certain limitations. It is also note worthy that profile of Yersinia enterocolitica isolates were seen to be similar within all the study areas. This suggest that all the herd investigated harboured common strain of Yersinia enterocolitica and in accordance with the findings of [20] .

Prevalence rates of 15% and 20% recorded for faecal and tonsil samples in our studies confirms previous studies that have identified this bacteria (Yersinia enterocolitica) more frequently in tonsils than in faeces [10,27,28,29,30,31] .

Exceptionally high susceptibility to spectrum of antibiotics such as ciprofloxacin, tarivid and gentamycin as documented in our finding has been similarly reported in Nigeria by [14,20] .

Our results support the assumption that the use of conventional cultivation methods may lead to considerable underestimation of pathogenic Yersinia enterocolitica in pork products/samples [9] . The reported rate of Yersinia enterocolitica isolation from faeces specimen is usually far below the actual incidence [21] . Highly Selective culture media such as CIN used in this study is highly recommended for future studies. This study did not take into consideration seasonal differences in occurrence of Yersinia. This may be necessary in future investigation.

Overall, our findings implicated tonsils as the most volunerable tissue to Yersinia enterocolitica infection [32] . Hence the removal of this tissue during the slaughter process should be considered in order to minimize the possibility of

contamination of meat products.

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Author Information

A.E.J. Okwori
Department of Medical Microbiology, Federal College of Veterinary and Medical Laboratory Technology

S.E. Agina
Department of Botany, University of Jos

A.O. Olabode
Department of Medical Microbiology, Federal College of Veterinary and Medical Laboratory Technology

A.A. Ochapa
Department of Medical Microbiology, Federal College of Veterinary and Medical Laboratory Technology

A.A. Turay
Department of Medical Laboratory Sciences, Ambrose Alli University

L.H. Lombin
National Veterinary Research Institute