



Isolation and characterization of pathogenic *Yersinia enterocolitica* from pigs in Abidjan, Côte d'Ivoire

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Originally Submitted on 2nd December 2011. Published online at www.m.elewa.org on February 27, 2012.

ABSTRACT:

Objective: To investigate the prevalence and to characterize of pathogenic *Yersinia enterocolitica* strains recovered from raw pig samples from a slaughterhouse in Abidjan, Côte d'Ivoire.

Methodology and Results: A total of 310 different pig samples from a slaughterhouse in Abidjan were examined by using pre-enrichment in trypticase soya broth followed by cold enrichment in phosphate buffered saline broth. Auliso's alkali treatment method was then performed before streaking onto MacConkey agar. Three pathogenic *Yersinia enterocolitica* strains were isolated from different pig samples. From tongue samples, *Y. enterocolitica* strains were isolated in 1% (2/200) and in 0.9% (1/110) of fecal samples. All the three strains were biotype 4, serotype O: 3 and phagetype VIII. The strains were negative for the pyrazinamidase test, lipase, esculin, and autoagglutination and not motile at 25°C. *Y. enterocolitica* strains showed multiple resistances to antibiotics such as sulfonamide and erythromycin.

Conclusion: *Y. enterocolitica* 4/O: 3/VIII strains were potentially pathogenic, representing a risk for the consumers in regard to yersiniosis in human health. This study showed that *Yersinia enterocolitica* biotype 4 serotype O: 3 phagetype VIII which is the most frequent cause of sporadic yersiniosis in Europe is present in Côte d'Ivoire and this bioserotype species comes from pigs, the main reservoir. Therefore, it is necessary to educate the public about the consumption of raw or undercooked pork. To our knowledge, this study is the first which shows the presence of *Yersinia* pathogen in pigs in Côte d'Ivoire. The results obtained in the present study could serve for future investigations of the *Yersinia enterocolitica* infection, mainly focusing on the possible contamination routes at the pork production and possibility of prevention at farm level.

Keywords: *Yersinia enterocolitica*, pathogenic, Pigs, tongue, fecal, prevalence, antibiotic.

INTRODUCTION

Among the *enterobacteraceae*, *Yersinia enterocolitica* is a foodborne pathogen causing a variety of symptoms in humans, ranging from mild diarrhea to immunological complications and potentially lethal septicaemia (Bottone, 1999). The most common manifestation is acute gastroenteritis, which particularly affects young

children (Tauxe et al., 1987; Verhaegen et al., 1998) 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 foodborne pathogen even though pathogenic isolates have seldom been recovered from foods (De Boer, 1995). *Y.*

enterocolitica is frequently harbored by healthy pigs which have been identified as a major reservoir of the human pathogenic *Yersinia* strains (Ramirez *et al.*, 2000). The transfer of pathogenic strains of *Y. enterocolitica* to humans occurs primarily during food consumption if hygienic rules are not kept during food processing and/or storage. Even chilled food (meat, milk, and vegetables) may pose a risk of infection because *Y. enterocolitica* strains can survive at low temperatures (- 4°C) (Fratamico *et al.*, 2005). In humans, *Y. enterocolitica* is usually transmitted by the ingestion of contaminated pork, milk or water. Virulent *Y. enterocolitica* strains are mainly isolated from pig tonsils, tongues, and carcasses (Baumgartner *et al.*, 2007; Simonova *et al.*, 2008). The pathogen resides mainly in the oral cavity (tonsils and tongues) and in the intestines (Lambertz and Danielsson-Tham, 2005). Pork and untreated fresh water are the most common sources of infection in humans (Ostroff *et al.*, 1994). In case-control studies, a correlation has been demonstrated between the consumption of raw or undercooked pork and yersiniosis (Ostroff *et al.*, 1994). Human yersiniosis in many European countries is the third most common enteric disease after campylobacteriosis and salmonellosis (EFSA, 2006). In spite of the severity and frequency of yersiniosis, knowledge about the occurrence of pathogenic *Y. enterocolitica* in pork products is limited. Appropriate analytical methods play a key role in the understanding of the epidemiology. In

general, and in particular in the light of Hazard Analysis Critical Control Point (HACCP), reliable and more rapid methods are needed for the detection of the bacterium in food and for the control of pork production. The methods available today based on culturing have several shortcomings such as inadequate differentiation between pathogenic and non-pathogenic strains and are being very laborious (Nesbakken, 1992). In animals' food, antimicrobials are used for control and treatment of bacterial associated infectious diseases as well as for growth promotion purpose (Phillip *et al.*, 2004). Therefore, the administration of antimicrobial agents for the treatment of bacterial infections in both veterinary and human medicine poses a potential risk because it leads to the selection of strains resistant to antibiotics. A high incidence of resistant bacteria has particularly been reported from developing countries, where antibiotics are freely available and their use is not subjected to any regulation (Levy, 1998). Although the occurrence of *Y. enterocolitica* in pigs has been investigated in several countries in particular in Africa, there is no report about the incidence of *Y. enterocolitica* in Côte d'Ivoire. So, the objectives of the present study were to investigate the prevalence and to characterize *Yersinia enterocolitica* strains isolated from pig sources in Côte d'Ivoire in order to determine their species, biotype, serotype and phagetype and evaluate their drug resistance profile and some other virulence characteristics.

MATERIAL ET METHODS

Sampling: From January 2009 to September 2010, a total of 310 samples were collected from apparently healthy pigs at a slaughterhouse in Abidjan, Côte d'Ivoire. The feces samples and tongues were swabbed with sterile cotton wool. The cotton swab samples were transferred into tubes containing 9 ml of Trypticase Soya Broth with Novobiocin (MERCK, Darmstadt, Germany). The samples were stored cold during transportation in an ice box at 4°C within 2 hours from collection and taken to the laboratory for immediate processing.

Isolation and identification of *Yersinia enterocolitica*: *Y. enterocolitica* strains was isolated using two stages enrichment procedures including pre-

enrichment in trypticase soya broth with Novobiocin (MERCK, Darmstadt, Germany) overnight at 28°C and selective enrichment using cold method (21 days at 4°C) in PSMB (Phosphate buffered saline supplemented with 1% mannitol, 1% sorbitol and 0.15% bile salts). 0.5 ml of TSB was transferred into 4.5 ml of PSMB, which was incubated at 4°C for 21 days. In order to reduce the background contaminating flora, Aulisio's alkali treatment method (Aulisio *et al.*, 1980) was performed and the immediate streaking onto MacConkey agar (Bio-Rad, Marnes-La-Coquette, France) supplemented with 1% sorbitol. The plates were incubated at 25°C for 48 h. After incubation, the plates were examined for characteristics colonies.

Biochemical properties: One to five small (diameter < 2 mm), transparent or pale pink colonies with characteristics of *Yersinia* were transferred onto one to five plates of trypticase soy agar (TSA, Oxoid, France) plates to create pure culture and incubated at 25°C for 24 h. All the isolates from pure culture were examined for Gram's staining, oxidase, catalase test, urease activity, tryptophane deaminase, glucose and lactose fermentation, gas formation from glucose, H₂S production, lysine decarboxylase, utilization of Simmons citrate, mannitol fermentation, reduction of nitrate and motility at 25°C and 37°C (ISO 10273:2003). The strains were further confirmed by the test of API 20E and API 50CH strips (BioMérieux, Marcy l'Etoile-France) incubated for 24 to 48 h at 25°C.

Serotyping and Phagetyping: All the strains were serotyped with 53 specific antisera. O antigens were determined by slide agglutination with the 53 difference antisera, according to the typing scheme of Wauters *et al.* (Wauters *et al.*, 1991). Phage typing was carried out with a set of 12 lysogenic phages and 16 sewage phages (Nicolle *et al.*, 1976).

Phenotypic virulence markers: Pyrazinamidase activity was tested at 28°C, and autoagglutination at

37°C in trypticase soya broth (Biokar Diagnostics; Beauvais, France) as previously described by Kandolo and Wauters (1985).

Antibiotic susceptibility testing: Antibiotic susceptibilities were determined by the disc diffusion method on Mueller-Hinton agar (Oxoid, France) according to the procedure of Bauer *et al.* (1966). The results were interpreted according to the criteria of the Comité de l'Antibiogramme of the French Society of Microbiology. The plates were incubated for 24-48 hrs at 37°C and resistance was scored via visual examination. The antimicrobial drugs tested and their concentrations on the discs (Bio-Rad, Marnes-La-Coquette, France) were as following: penicillin (10UI), amoxicillin (25 µg), ampicillin (10µg), amoxicillin/clavulanic acid (20 µg/10 µg), ticarcillin (75 µg), imipenem (10µg), cefalothin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ceftazidime (30µg), aztreonam (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), kanamycin (30µg), gentamicin (15 µg), amikacin (30µg), chloramphenicol (30µg), trimethoprim/sulfamethoxazole (1.25/23.75µg), sulfonamide (200 µg), erythromycin (15 µg) and tetracycline (30 UI).

RESULTS

Sampling and isolation: A total of 310 pig samples, comprising 200 pig tongues swab samples and 110 feces swab samples from slaughterhouse in Abidjan from different pig farms in Côte d'Ivoire were analyzed.

The samples were tested for the presence of *Yersinia enterocolitica*. *Y. enterocolitica* strains were isolated in 1% of tongue and 0.9% of fecal samples (**Table 1**).

Table 1: Prevalence of pathogenic *Y. enterocolitica* in pig samples

Sources	Number of samples	No. (%) of pathogenic <i>Y. enterocolitica</i> positive samples
Tongue	200	2 (1)
Feces	110	1(0.9)
Total	310	3(1)

Biochemical properties: All the strains showed positive reactions for catalase, nitrate reduction, β-galactosidase, ornithine decarboxylase, Urease, D-glucose, D-mannitol, inositol, D-sorbitol, D-sucrose, amygdalin and L-arabinose. The following tests revealed negative reactions: Gram's staining, oxidase, tryptophane deaminase, indole production, lysine decarboxylase, lysine deaminase, citrate utilization, H₂S production, arginine dihydrolase, Voges-

Prauskauer, gelatinase, lactose, L-rhamnose, D-melibiose, gas formation from glucose, pyrazinamidase activity, lipase, agglutination at 37°C, motility at 25°C and motility at 37°C.

The three strains of *Y. enterocolitica* isolated from pigs were examined for antibiotic susceptibility. Susceptibility to twenty one antibiotics is shown in table 3.

Table 2: Scheme used for biotype and phenotypic characteristic of *Yersinia enterocolitica* isolated from pigs as described in ISO 10273:2003, according to wauters (Wauters et al., 1991) and to Kandolo and Wauters (1985).

Ref	IP No	Source	Pyr	Lip	Esc	Ind	Treh	Autoag	Biotype	serotype	Phagetype
L274	33925	Tongue	-	-	-	-	+	-	4	O:3	VIII
L275	33926	Tongue	-	-	-	-	+	-	4	O:3	VIII
L276	33927	Feces	-	-	-	-	+	-	4	O:3	VIII

Ref, reference; IP No, Institute Pasteur number; Pyr, pyrazinamidase; Lip, lipase; Esc, esculin; Ind, indole treh, trehalose; Autoag, Autoagglutination.

Table 3: Antibiotic susceptibilities of *Yersinia enterocolitica* isolated from samples of pigs

Antibiotic	Disc potency	<i>Y. enterocolitica</i> (3)		
		Number (%) of isolates		
		Resistant	Intermediate	Sensitive
Penicillin	10UI	3 (100)	0	0
Amoxicillin	25µg	3(100)	0	0
Ampicillin	10µg	3 (100)	0	0
Amoxicillin/ clavulanic acid	20/10µg	2(66.7)	1 (33.3)	0
Ticarcillin	75µg	3(100)	0	0
Imipenem	10µg	0	0	3 (100)
Cefalothin	30µg	3 (100)	0	0
Cefoxitin	30µg	0	0	3 (100)
Ceftriaxone	30µg	0	0	3(100)
Ceftazidime	30µg	0	0	3(100)
Aztreonam	30µg	0	0	3(100)
Erythromycin	15 µg	3 (100)	0	0
Nalidixic acid	30µg	0	0	3 (100)
Ciprofloxacin	5µg	0	0	3(100)
Kanamycin	30µg	0	0	3 (100)
Gentamicin	15 µg	0	0	3 (100)
Amikacin	30µg	0	0	3 (100)
Chloramphenicol	30µg	0	0	3 (100)
Sulfonamide	200 µg	3 (100)	0	0
Trimethoprim/sulfamethoxazole	1.25/ 23.75µg	0	0	3 (100)
Tetracycline	30UI	0	0	3 (100)

DISCUSSION

Yersinia enterocolitica is a major cause of foodborne disease in developed countries mainly in temperate zones. Studies concerning the presence of enteropathogenic *Y. enterocolitica* in foods and humans are rare in developing countries and tropical areas. Pigs were studied in Abidjan of Côte d'Ivoire in order to obtain among the different tested pigs slaughtered, varied information on the presence of enteropathogenic *Yersinia*.

In spite of the limited number of samples examined, this report establishes the presence of *Y. enterocolitica* in raw pork in Côte d'Ivoire. In another study, Fredricksson-Ahomaa et al. (2001) recovered pathogenic *Y. enterocolitica* strains from all positive

fecal samples after overnight enrichment in TSB. Thus, it appeared that some enrichment procedures were better than others (direct plating, cold enrichment, new enrichment medium, enrichment at different temperatures and days). For example, Jiang et al. (2000) reported that the highest isolation rate of *Y. enterocolitica* was 32% for the 4°C/3-week enrichment, followed by 28% for the 4°C/2-week enrichment, 26% for the 25°C/24-h enrichment, 22% for the 4°C/1-week enrichment, and 10% for direct plating. Therefore, the isolation ratio may depend on the procedure. However, nonpathogenic isolates and other psychrotrophic bacteria also multiply during cold enrichment. Thereby, in Belgium, most laboratories have stopped using cold

enrichment since it also increases the isolation of nonpathogenic *Y. enterocolitica* strains (Verhaegen *et al.*, 1998). Furthermore, the identification of *Y. enterocolitica* by traditional agar plate techniques (ISO 10273:2003) is complicated by the fact that on the commonly used selective agar plates, especially the cefsulodin-irgasan-novobiocin (CIN) agar has several unrelated bacteria also growing (Arnold *et al.*, 2004). In addition, some *Yersinia* strains are inhibited by CIN agar (Fukushima and Gomyoda, 1986).

In all the samples examined 3 pathogenic strains of *Yersinia enterocolitica* were identified. To our knowledge, it was the first time *Y. enterocolitica* was isolated in Côte d'Ivoire. In this investigation, *Y. enterocolitica* 4/O: 3 strains were isolated in 1% of tongue and 0.9% of fecal samples. *Y. enterocolitica* strains were isolated more frequently in tongue than fecal samples. All the three strains of *Y. enterocolitica* belong to the biotype 4, serotype 3, phagetype VIII. In others studies, Novoslavskij *et al.* (2010) reported that the prevalence of *Y. enterocolitica* among different tested pig herds varied 0%-70% in feces and 0%-60% in carcass swab samples. Despite the fact that, it is known that the isolation rate of *Y. enterocolitica* is higher in countries with cold climate, this study has emphasized the presence of pathogenic *Y. enterocolitica* in Côte d'Ivoire. Thus, the prevalence of *Y. enterocolitica* among different tested pig samples from slaughterhouse was 1%. In Nigeria also, bioserotype 4/O: 3 was found in the fecal samples of one pig and one sheep (Okwori *et al.*, 2009). Likewise, in South Africa, *Y. enterocolitica* was isolated in 1% of 1634 fecal examinations (Jennings *et al.*, 1987) and Rabson and Koornhof (1972) examined the tools and mesenteric lymph nodes of 200 randomly selected pigs at the Johannesburg abattoirs and isolated one (0.5%) *Y. enterocolitica* of these animals. Unlike of this study, some researchers reported that no *Y. enterocolitica* strains were isolated in their study. For instance, in Senegal, Chambron and Bourdin (1971) and Franzin *et al.* (1987) did not recover *Y. enterocolitica* in 137 pigs and 108 patients with gastrointestinal disorders, respectively. Otherwise, the prevalence of bioserotype *Y. enterocolitica* 4/O: 3 strains in slaughter pigs has been reported to be 56% in Finland (Korte *et al.*, 2004) and 60% in southern Germany (Fredriksson-Ahomaa *et al.*, 2001a). Also, in Norwegian study investigating, Johannessen *et al.* (2000) monitoring the occurrence of *Y. enterocolitica* pathogenic strains in 249 pig samples from five different slaughterhouses, found in 15.2% of cases the pathogenic strains of *Y. enterocolitica* O: 3.

The low rate of our isolation of pathogenic *Y. enterocolitica* in samples may be due to the limited sensitivity culture methods. The apparently low prevalence of pathogenic *Y. enterocolitica* in food may be due to lack of suitable selective methods, as reported by Magras *et al.* (2008). For Fredricksson-Ahomaa and Korkeala (2003), there are difficulties associated with the isolation of pathogenic *Y. enterocolitica* strains from the small number of pathogenic strains in the samples and the large number of organisms in the background flora, especially in food and environmental samples. The differences between the findings of various authors and those of this study might be due to several factor such as isolation methods, number of analyzed samples, season, and geographical location. These factors may cause an increase or decrease in the prevalence of the *Yersinia spp.* (Siriken, 2004). In addition, the present study was performed in Abidjan, where the climate is generally warm. Authors suggested that the contamination derived probably from slaughtered animals. In this study, the low prevalence of pathogenic *Y. enterocolitica* observed in the pig slaughtered samples suggests that slaughterhouse does not represent the main source of contamination of pigs by human pathogenic *Y. enterocolitica*. Rather, transmission is more likely from others infected pigs.

The results of antimicrobial resistance showed that the strains of *Y. enterocolitica* presented multiple antibiotic resistances as to ampicillin, amoxicillin, amoxicillin/clavulanic acid, cefalothin, penicillin, erythromycin, ticarcillin and sulfonamide. In early previous study, Baumgartner *et al.* (2007) reported also that there were high level of resistance to ampicillin, cefalothin and amoxicillin/clavulanic acid. Less than 10% of clinical isolates and strains from pig feces were resistant to streptomycin, sulfonamide, trimethoprim/sulfamethoxazole, tetracycline, trimethoprim and chloramphenicol, but strains from retail pork were all susceptible to these antimicrobial agents. In another study, Kwaga and Iversen (1990) showed that all or most of the *Yersinia* strains were resistance to ampicillin, penicillin, cefalothin and amoxicillin/clavulanic acid while 100% of them were susceptible to trimethoprim/sulfamethoxazole and imipenem. In this present study, one strain's (L276) resistance was intermediate to amoxicillin/clavulanic acid. These and related drugs are used in veterinary medicine to treat swine (Prescott and Baggot, 1988) which may account for the increase in resistance. Since the resistance to drugs encountered in strains of *Y.*

enterocolitica isolated from humans may originate in animal through a process of selection (Trallero et al., 1988), there is a need to monitor continuously the profile of resistance of animal and food isolates.

All the strains of *Y. enterocolitica* tested by pyrazinamidase activity were negative. So, this present study *Y. enterocolitica* isolates should be considered as pathogenic strains. Likewise, the biotyping, serotyping and phagetyping of isolates can be helpful in determining whether they are potential pathogens. In this present study, the biotyping, serotyping and phagetyping of *Y. enterocolitica* isolates were performed and so, all the three strains of *Yersinia enterocolitica* belong to the biotype 4, serotype 3, phagetype VIII (100%). Fredricksson-Ahomaa et al. (2006) reported that healthy pigs are often carriers of strains of *Yersinia enterocolitica* that are pathogenic to humans, in particular bioserotype O: 3/4 strains. Pigs are the only reservoir from which *Y. enterocolitica* 4/O: 3 strains have been frequently isolated. The organisms are present in the oral cavity, particularly on the tongue and in the tonsils, and in the intestine and feces. This is probably the result of spread of the organism via feces,

CONCLUSION

This study showed that *Yersinia enterocolitica* biotype 4 serotype O:3 phagetype VIII which is the most frequent cause of sporadic yersiniosis in Europe is present in Côte d'Ivoire and this bioserotype species come from pigs, the main reservoir. The occurrence of virulent strains of *Y. enterocolitica* shows that the pigs are potential sources of human infection by these bacteria in Côte d'Ivoire. Virulent strains of *Y. enterocolitica* were found, with biotype, serotype and phagetype even though in low percentage and thereby represented a

ACKNOWLEDGEMENTS

We thank Dr. Elisabeth CARNIEL (Centre National des Yersinia, Institut Pasteur, Paris, France) for her kind cooperation, her generous advice and for confirming of strains characteristic (biotype, serotype and phagetype) of the *Yersinia enterocolitica* strains. The

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intestinal contents, or contamination from the oral cavity during slaughter and dressing operations. Biotype 4/O: 3 is generally the predominant bioserotype in pig production systems (Laukkanen et al. 2009, Van Damme et al. 2010). In Europe, the majority of human pathogenic *Y. enterocolitica* belongs to biotype 4 (serotype O: 3), followed by biotype 2 (serotype O: 9) (EFSA, 2009). In France for example, biotype 4 is the most prevalent biotype among the strains isolated from humans (69%), followed by biotype 2 (30%) and biotype 3 (1%) (Savin and Carniel, 2008). Also, Tauxe et al. (1987) in Belgium and Ostroff et al. (1994) in Norway reported that the association between yersiniosis in man and the consumption of pork and identified raw or undercooked pork as main source of infection. Therefore in Belgium, the country with the highest incident of yersiniosis, *Yersinia enterocolitica* O: 3 infections have been linked to the ingestion of raw pork (Tauxe et al., 1987). In a case-control study, Jones et al. (2003) showed also that yersiniosis clearly was associated with chitterlings (boiled pig small intestines).

risk for the consumers in regard to yersiniosis. Therefore, it is necessary to educate the public about the consumption of raw or undercooked pork. This is the first report of the pathogenic *Y. enterocolitica* in pig sources in Côte d'Ivoire. The results obtained in the present study could serve for future investigations on *Yersinia enterocolitica*, mainly focusing on the possible contamination routes at the pork production and possibility of prevention at farm level.

slaughterhouse of SIVAC (Société Ivoirienne d'Abattage et de Charcuterie, zone industrielle, Yopougon, Abidjan) is acknowledged for their cooperation in sampling.

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