

Prevalence and Diversity of *Pseudomonas* spp. Isolated from Beef, Fresh and Smoked Fish in Abidjan, Côte d'Ivoire

Benie CKD^{1,2*}, Dadie A^{1,2}, Guessennd NK^{2,3}, Kouame ND¹, Yobouet BA^{1,4}, Aka S^{1,4}, Koffi MD¹ and Dosso M^{2,3}

¹University of Nangui-Abrogoua, Abidjan, Côte d'Ivoire, Laboratory of Biotechnology and Food Microbiology (LMBM), Abidjan, Cote d'Ivoire

²Department of Bacteriology and Virology, Institute Pasteur of Côte d'Ivoire (IPCI), Abidjan, Côte d'Ivoire

³University of Félix Houphouët Boigny, Faculty of Medical Sciences, Abidjan, Côte d'Ivoire

⁴Research and Development of Swiss Center for Scientific Research in Côte d'Ivoire, Abidjan, Côte d'Ivoire

Research Article

Received Date: 01/12/2016

Accepted Date: 06/12/2016

Published Date: 12/12/2016

*For Correspondence

Comoé Koffi Donatien Benie, Department of Bacteriology and Virology, Institute Pasteur of Côte d'Ivoire (IPCI), 01 BP 490 Abidjan 01, Côte d'Ivoire, Tel: +22507555844/+22505206444.

E-mail: dona.comoe@yahoo.fr

Keywords: *Pseudomonas*, Beef, Fish, Serogroups, Côte d'Ivoire

ABSTRACT

Pseudomonas is an agent of food spoiling, but its role in the contamination of certain local products is unknown. The objective of this study was to evaluate the diversity and the prevalence of *Pseudomonas* species contaminating beef, fresh and smoked fish sold in some markets. A total of 182 samples including 95 beef, 41 fresh fish and 46 smoked fish were collected and analyzed. The Cetrimide Fucidin Cefaloridine (CFC) Base *Pseudomonas* agar and Cétrimide agar were used for *Pseudomonas* spp. isolation according to the ISO 6887-2 standard method. Strains were first characterized by API 20NE, then confirmed by polymerase Chain Reaction (PCR), using 16S rRNA and *rpoB* genes. Serotyping of the strains of *P. aeruginosa* was done by a slide agglutination test. The prevalence of *Pseudomonas* was 97.9%, 87.8% and 63.1% respectively in beef, in fresh fish and in smoked fish. A total of 158 (97.5%) isolates were confirmed as *Pseudomonas* by 16S rRNA. The prevalence of majority species was 46.8% for *P. aeruginosa*, 26.6% for *P. putida* and 8.2% for *P. fluorescens*. The prevalence of the majority of *P. aeruginosa* serogroups was 20.3%, 14.9%, 12.2% and 12.2% respectively for O11, O5, O16 and O7 serogroups. The study revealed a high prevalence of *P. aeruginosa* serogroups which represent a risk for human health. It requires improving the health management of the food chain of the products analyzed to prevent their possible alteration or reduce the risk of infections.

INTRODUCTION

Pseudomonas, mainly *P. aeruginosa* is a major agent associated with nosocomial infections and food poisoning [1-3]. There is an emergence of species of the *Pseudomonas* spp. which express various virulence determinants [4,5]. Among *Pseudomonas*, *P. aeruginosa* was known to be dangerous. In fact, *P. aeruginosa* form biofilms who confer a high power colonization of food spoilage, resistance to antiseptics, disinfectants and antibiotics [6-8]. The prevalence of *P. aeruginosa* infections was 11.5% in Europe and 17% in developing countries. Food industries accord a particularity to *P. aeruginosa* because of its ability to grow rapidly and form biofilms [2].

In human being *Pseudomonas* are also isolated. The most frequently isolated species are *P. aeruginosa*, *P. fluorescens*, *P. putida* and *P. stutzeri* [9]. Overall mortality related to *P. aeruginosa* was also evaluated up to 70% and the mortality rate is about 40% [5,10]. Many factors are used by *P. aeruginosa* to make virulence. We note lipopolysaccharide (LPS) which is an important virulence factor has endotoxic effects. The nature of the LPS molecule including a hydrophobic lipid part, a central core oligosaccharide and another part of polysaccharide defined as O antigen or O polysaccharide [2,4] were the main factors implicated into virulence. The current epidemiological data indicated that most of the 20 serotypes of International Antigenic Scheme (IATS), IATS-O1,

serogroups 2 (IATS-O2, IATS-O5 and IATS-O16), IATS-O6, and IATS-O11 are responsible for 70% of *P. aeruginosa* infections [2,11]. The relationship between virulence of *P. aeruginosa* and different serogroups has been studied [2,11,12].

Strains having any of toxins secreted by the type III secretion system (TTSS), exotoxin (ExoU) were often as 11 serotypes, whereas 6 serotype strains have been associated with a phenotype of exotoxin U (ExoU) negative [13]. Moreover, it has been reported that some *P. aeruginosa* serogroups are able to induce high resistance to antibiotics [7,12,13].

Pseudomonas, because of their ubiquitous character, could live in very hard ecological niches [14]. They can be isolated from food such as; milk, dairy products, meat, fish and seafood [3,15-17]. *Pseudomonas* bacteria are psychrotrophic indicators of spoilage of the meat and can grow thereon at room temperature between 0°C and 30°C [18]. Meat and fish are important in the diet of people of West Africa [19-21]. They also provide a valuable supplement in diets low in protein, essential vitamins and minerals [22]. Animal products (meat, fish) contribute significantly to food security, nutrition and poverty reduction [19,23].

In Côte d'Ivoire, fishing and breeding hold a relatively important place in the national socio-economic balance because; they live about 500,000 people and accounts for 3% of Gross domestic product (GDP). However, despite the high nutritional value of fish and meat, they are highly perishable food and face enormous constraints that limit their productivity and competitiveness. Conservation mode, the different ways of smoking fish and selling conditions as well as slaughtering, cutting and distribution of beef carcasses may cause product contamination. If some work was done on the characterization of *Pseudomonas* linked to nosocomial infections and the environment [15,24] the data are scarce or nonexistent in the prevalence and diversity of *Pseudomonas* strains contaminating animal products.

The aim of this study was to evaluate the prevalence and diversity of the genus *Pseudomonas spp.* isolated from beef, fresh fish and smoked fish sold in Abidjan, Côte d'Ivoire.

MATERIALS AND METHODS

Materials

Beef, fresh fish and smoked fish were used as biological material. Fish were consisting of horse mackerel (*Atlantic horse mackerel*); sardine (*Sardina pilchardus*) and carp (*Cyprinus carpio*). *Pseudomonas aeruginosa* ATCC 27853 was used as reference strain for quality control.

Study site

The cross- type study was conducted in the district of Abidjan, the economic capital of Côte d'Ivoire and located on the Ebrié lagoon, in the south of the country (**Figure 1**). Abidjan has chosen because, it is one of the original areas of high production and consumption of beef and fish. Also Abidjan has most of 5 billion of people who consume lot of beef and fish. The study was conducted in five districts such as Port-Bouet, Abobo, Yopougon, Adjame and Bingerville. This stud was led in these districts according to their importance in the production and sale of beef and fish and consent of the manufacturer and merchants to participate in the study.

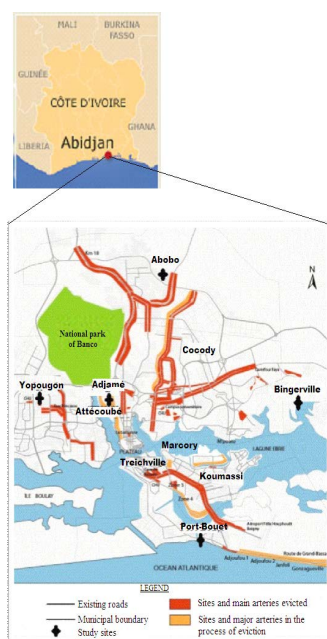


Figure 1. Map of Abidjan with materialization of study sit.

Sampling

The study focused on beef, fresh fish and smoked fish. Fish were made of horse mackerel (*Atlantic horse mackerel*); sardine (*Sardina pilchardus*) and carp (*Cyprinus carpio*). The samples were collected from January to April 2015. The beef was taken in the markets of sale and in slaughterhouses. Fresh fish and smoked were taken primarily on the stalls in the markets. Fresh fish was also taken to Abidjan fishing port. A total of 182 samples including 95 samples of beef, 41 samples of fresh fish and 46 samples of smoked fish were taken. Two hundred grams of each sample were taken and put in Stomacher bags. All samples collected were immediately stored at +4°C in a cooler containing carboglaces and transported to the laboratory for analyzes. Analyzes were performed within two hours following sampling.

Isolation and Enumeration of *Pseudomonas* spp. in Food Matrices

A quantity of 10 g from each sample of beef, fresh fish and smoked fish was taken and mixed in 90 ml of sterile peptone water (Oxoid LTD, England) in a polyethylene bag and mixed with stomacher 400 (Colworth, England) for 2 min. 1 ml of each sample was taken and added to 9 ml of trypticase soy broth (TCS) (Bio-rad France). Samples were serially diluted with sterile peptone water 0.1% (Difco) (10^{-1} to 10^{-4}) [25] (NF EN ISO 6887, 2004) and surface plated with 0.1 ml in duplicate CFC (Cetrimide, fucidin, cephaloridin) *Pseudomonas* base agar (Oxoid, Code: 0559, England) supplemented with SR0103 (Oxoid, Hants, England) and cetrimide agar (Biokar, Paris, France). *Pseudomonas* CFC agar base supplemented with SR0103 was used for isolation of *Pseudomonas* spp. while, cetrimide agar was used for the isolation of *Pseudomonas aeruginosa*.

On CFC *Pseudomonas* base agar supplemented with additional SR103, *Pseudomonas* sp colonies are mucous, greyish, pigmented or not. Then, on cétrimide agar, presumptive *Pseudomonas aeruginosa* isolated were colonies producing blue-green, brown or fluorescence pigmentation. After 18-24 hours (+/- 1 h) of incubation at 37°C, the Petri dishes containing 30 to 300 colonies were selected and colonies were counted according to ISO 13720 standard methods [26,27] (ISO 13720, 2010). ISO 7218, (2007) was used to estimate and performed *Pseudomonas* colonies.

Identification of *Pseudomonas*

Five presumptive colonies of *Pseudomonas* (as describe in isolation and enumeration of *Pseudomonas* spp. in food matrices) were took on CFC *Pseudomonas* base and cetrimide agar. They were streaked on nutrient agar and incubated at 37°C for 24 h (+/- 1 h) for sub culture. Each of *Pseudomonas* spp. and *Pseudomonas aeruginosa* were confirmed with both of King A and King B agar (Biokar Diagnostics, BM10508, France) incubated respectively at 41°C and 37°C for 24 h. The production of specific pigments allowed the differentiation of *Pseudomonas aeruginosa* and *Pseudomonas* sp. Thus, production of fluorescent yellow-green pigment (pyoverdine) specific for *Pseudomonas* spp was highlighted on King B medium and producing a non-fluorescent blue pigment (pyocyanine) specific for *Pseudomonas aeruginosa* on King A.

API 20 NE strips (BioMérieux SA, Marcy l'Etoile, France) were used for a preliminary biochemical characterisation of the strains and the species were identified using the API database.

Molecular Characterization of *Pseudomonas* by 16S rRNA and *rpoB* Genes Detection

Template DNA was prepared by boiling 200µl of bacterial suspension in MilliQ ($OD_{600}=0.6$) in safe-lock Eppendorf tubes for 10 min. The tubes were immediately cooled on ice for 5 min and centrifuged ($20.000\text{ g} \times 10\text{ min}, 5^{\circ}\text{C}$). The supernatants were taken and subsequently kept at -20°C and used as template DNA for PCR studying.

Single PCR for characterization of each gene were carried out with 2 µl template DNA, 0.1 µl of each primer (20 mM) (Intégral DNA Technologie, France), 0.2 µl of deoxyribonucleoside triphosphates (10 mM), 5 µl of 5X PCR buffer, 1.5 µl of $MgCl_2$ (2 mM) and 0.5 U Prime Taq DNA polymerase (Promega Corporation) in a total volume of 25 µl.

DNA 16S region amplification was performed using the primer set 16SF-16SR [3] (16SF 5'-AGAGTTTGATCCTGGCTCAG-3'; 16SR 5'-CTACGGCTACCTGTTACGA-3') and the following thermal profile was 2 min at 94°C; 5 cycles consisting of 94°C for 45 s, 55°C for 1 min, 72°C for 2 min; 35 cycles consisting of 92°C for 45 s, 60°C for 45 s, 72°C for 2 min; final extension of 72°C for 2 min; and final cooling at 4°C. DNA *rpoB* region amplification was performed using the primer set *rpoB* F' (5'-CAGTTCATGGACCAGAACAACCCG-3') and *rpoB* R' (5'-ACGCTGGTTGATGCAGGTGTTTC-3'), aligning on positions 1552 and 2298 of the *rpoB* gene sequence of *Pseudomonas aeruginosa* UCBPP-PA14 (CP000438).

The *rpoB* DNA was amplified using the following protocol: initial denaturation (94°C for 3 min), followed by 30 cycles of denaturation (94°C for 1 min), annealing (58°C for 1 min) and extension (72°C for 2 min), with a single final extension of 7 min at 72°C. Amplification was performed with 10 µl of PCR products which were separated in 1.5% agarose gel for 30 min at 120 V. After amplification, the agarose gel was put in ethidium bromide (0.5 µg/ml) and detected by Molecular Imager Gel Doc™ EZ (Bio-Rad, USA).

Serotyping of *Pseudomonas aeruginosa* Isolates

The O-serotypes were determined by a slide agglutination test using four pools (OMA, OMC, OME, and OMF) and 20 monovalent antisera, O1 to O20 (Sanofi Diagnostics Pasteur), according to the manufacturer's recommendations.

Statistical Analysis

The data was entered with the data processing software SPSS Statistics 20.0 (IBM Corporation, SPSS Inc, Chicago, USA) and transferred to Excel. Statistical analyzes were performed with the software XLSTAT 2014.1 and Ri386 3.0.1 software. The descriptive statistics (frequency, mean, standard deviation) were used for quantitative variables. Geometric means were used to calculate counts. Counts were converted into base-10 logarithm (log10) in order to normalize the distributions.

Chi square test was used to test the relationship between variables. The difference between variables was considered significant at p<0.05.

To show the clonal relationship between serotype and the food matrix, a Principal Component Analysis (PCA) followed Ascending Hierarchical Classification (AHC) was performed. For AHC, the Ward criterion for hierarchical analysis was used.

RESULTS

Prevalence of Pseudomonas in Various Animal Products

According to **Table 1**, the overall prevalence of *Pseudomonas* in animal products was 86.8%. It is higher at the beef with 97.9%, followed by 87.8% with fresh fish. The lowest prevalence was found in smoked fish with 63.1%.

Table 1. Prevalence of *Pseudomonas* in various animal products.

Animals products	Number of samples	Strains of <i>Pseudomonas</i>	<i>Pseudomonas</i> prevalence (%)
Beef	95	93	97.9
Fresh fish	41	36	87.8
Smoked fish	46	29	63.1
Total	182	158	86.8

Molecular Identification of Pseudomonas

Molecular identification of presumptive strain from the 16S rRNA gene confirmed their belonging to the *Pseudomonas* (**Table 2**). Among 162 presumptive isolates of *Pseudomonas*, 158 (97.5%) were confirmed positive by PCR amplification of a fragment of 1351 bp of the 16S rRNA gene (**Figure 2a**). However 74 species of *P. aeruginosa* identified by API20NE were confirmed by *rpoB* gene following PCR methods (**Figure 2b**).

Table 2. Frequency of strains confirmed by the 16S rRNA and *rpoB* genes.

Genes	Number of isolates Presumptive <i>Pseudomonas</i> N=162		
	Confirmed species	Effective	percentage
16S rRNA	<i>Pseudomonas</i> spp.	158	97.5
<i>rpoB</i>	<i>Pseudomonas aeruginosa</i>	74	46.8

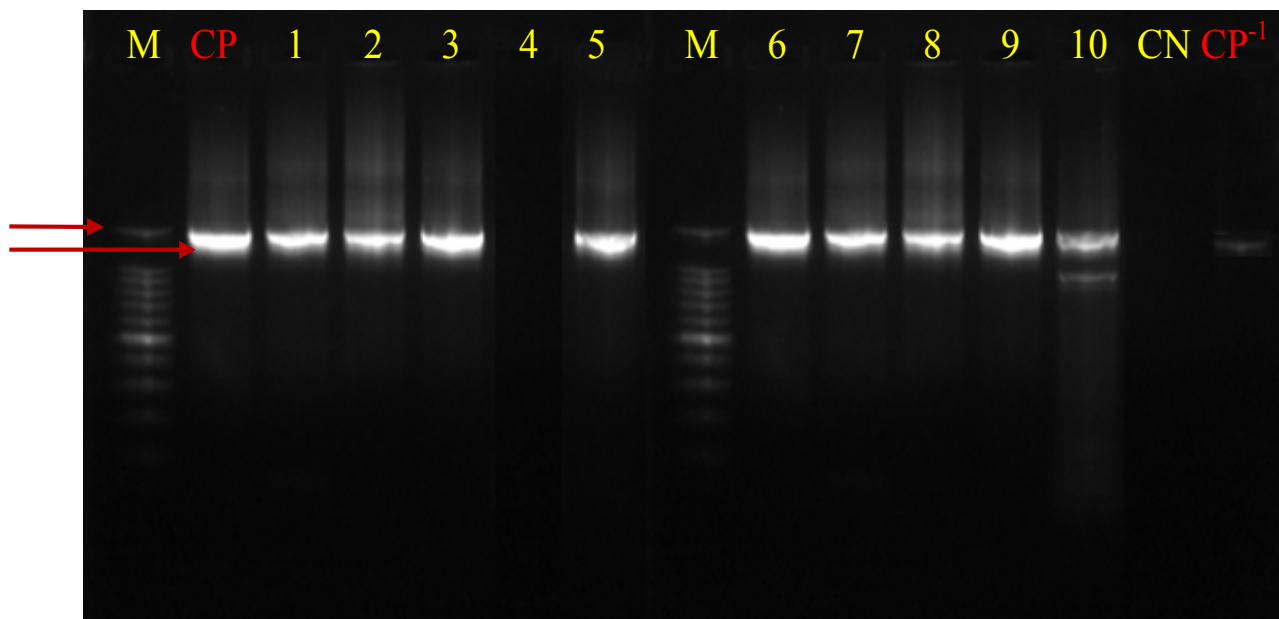


Figure 2a. Profile of the amplification product of the 16S rRNA gene of *Pseudomonas* genus.

CP: Positive control, *Pseudomonas aeruginosa* ATCC 27853; CN: Negative control; M: Marker Gene Ruler 100 bp (Bench Top, 100 bp DNA Ladder, Promega Corporation, USA).

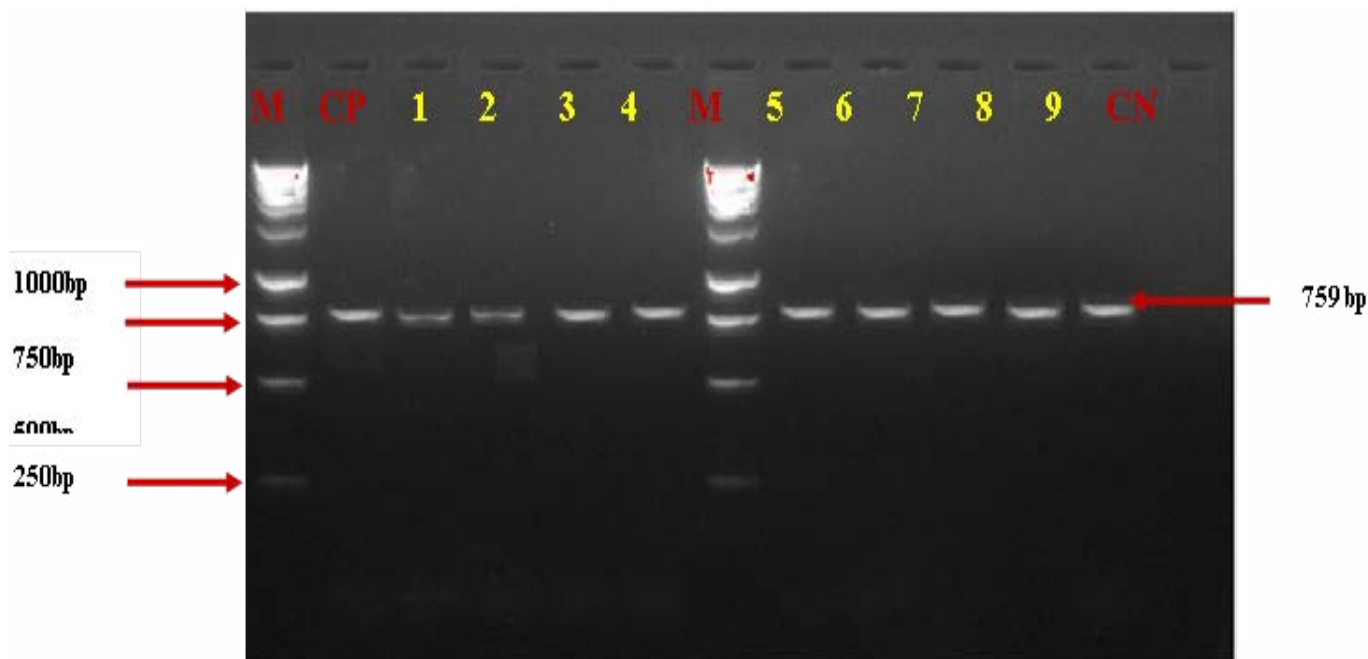


Figure 2b. Profile of the amplification product of the *rpoB* gene of *Pseudomonas aeruginosa*.

CP: Positive control, *Pseudomonas aeruginosa* ATCC 27853; CN: Negative control; M: Marker Gene Ruler 250 bp (Bench Top, 1 kb DNA Ladder, Promega Corporation, USA).

Diversity of Pseudomonas Isolated from Various Animal Product

The **Table 3** showed the diversities of *Pseudomonas* and their species isolated in this study. *P. aeruginosa*, are more isolated than others with 46.8% following by *P. putida* 26.6% and *P. fluorescens* with 8.2%. *P. dimunita*, *P. stutzeri*, *P. chlororaphis* and *P. putrefaciens* were also isolated at frequencies below 2%.

Table 3. Diversity of *Pseudomonas* isolated from various animal products.

Species of Pseudomonas	Different species of Pseudomonas isolated from animal products	
	Number of strains (N)	Frequency (%)
<i>P. aeruginosa</i>	74	46.8
<i>P. putida</i>	42	26.6
<i>P. fluorescens</i>	13	8.2
<i>P. cepacia</i>	10	6.3
<i>P. dimunita</i>	2	1.3
<i>P. stutzeri</i>	1	0.6
<i>P.chlororaphis</i>	1	0.6
<i>P.putrefaciens</i>	1	0.6
<i>Pseudomonas. sp</i>	14	8.9
Total	158	100

Prevalence of Pseudomonas Species by Animal Product

Pseudomonas aeruginosa prevalence (29.7%) found in all animal product is higher in beef than fresh fish (10.1%) and smoked fish (6.9%) (**Figure 3**). The study has also showed that the prevalence of *P. putida* in beef was 24.1% and was higher than the prevalence of *P. fluorescens* (4.4%). The prevalence of *P. cepacia* in beef, fresh fish and smoked fish is less than 4%. *Pseudomonas chlororaphis* was only isolated in smoked fish with a prevalence of 0.6%. While *Pseudomonas stutzeri* and *P.*

putrefaciens were identified only fresh fish with the same prevalence (0.6%) (Figure 3).

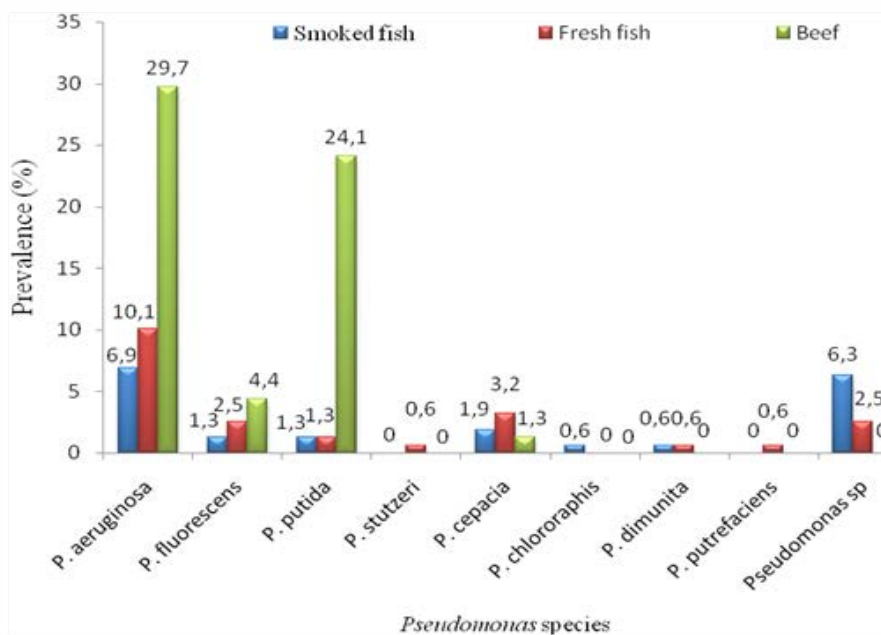


Figure 3. Prevalence of *Pseudomonas* species by animal product.

Serogroups Prevalence of *Pseudomonas aeruginosa*

Serogroups O11 with a prevalence of 20.3% was mostly isolated, followed by the serogroups O5 with 14.9%. However serogroups O16 and O7 have the same prevalence (12.2%). While, serogroups O8, O9, O15, O1, O10, O12, O2 and O4 have prevalence rates below 7% (Table 4). Serogroups O11 and O5 were isolated in beef, in fresh fish and in smoked fish with prevalence ranging from 2.7 to 12.2% (Figure 4). O7 and O16 serogroups were isolated from both beef and fresh fish with prevalence ranging from 2.7 to 9.5%. The serogroups O9 and O12 were isolated from both beef and smoked fish with prevalence ranging from 2.7 to 9.5%. The study showed that serogroups O1, O4, O8 and O15 were isolated only beef with a prevalence ranging from 2.7 to 6.8% (Figure 4). Also, the serogroups O10 and O2 were isolated only fresh fish and smoked fish with prevalence of 2.7 and 1.4%.

Table 4. Serogroups diversity of *Pseudomonas aeruginosa* isolated from animal products.

<i>Pseudomonas aeruginosa</i> serogroups		
Serogroups	Number (n = 74)	Prevalence (%)
O ₁₁	15	20.3
O ₅	11	14.9
O ₁₆	9	12.2
O ₇	9	12.2
SN	8	10.8
O ₈	5	6.8
O ₉	5	6.8
O ₁₅	4	5.4
O ₁	2	2.7
O ₁₀	2	2.7
O ₁₂	2	2.7
O ₂	1	1.4
O ₄	1	1.4

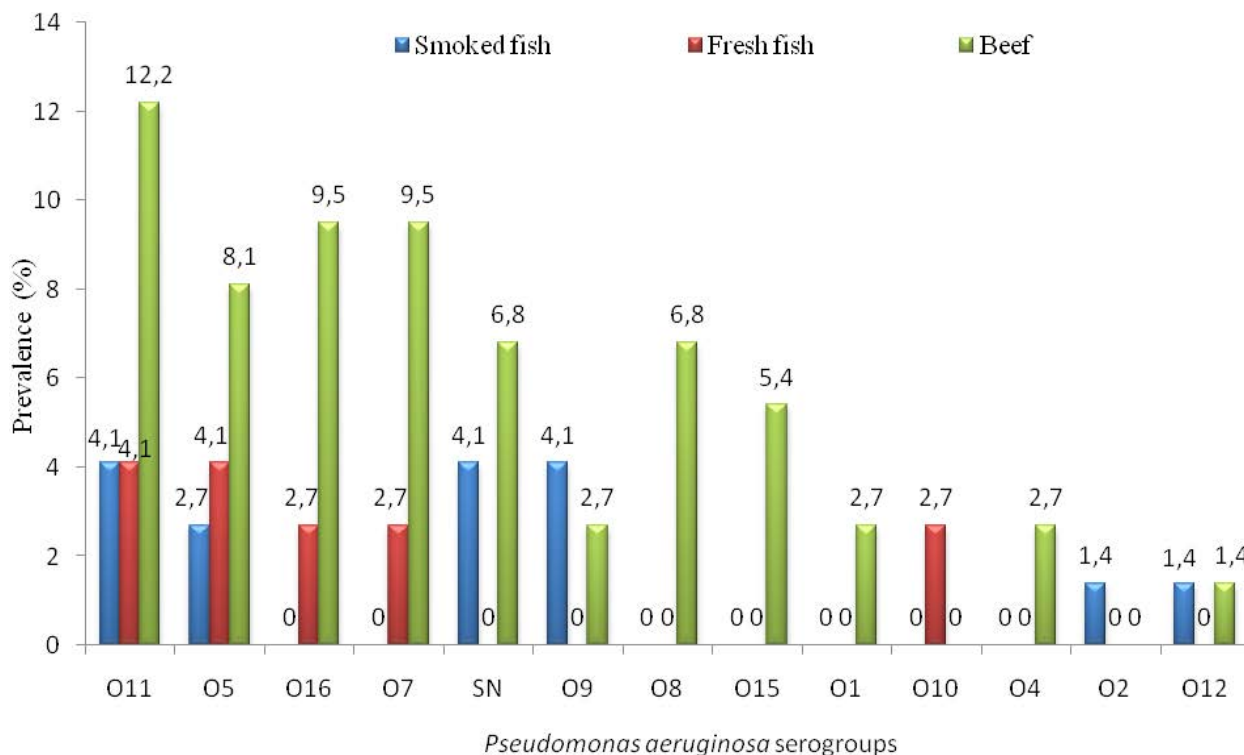


Figure 4. Serogroups prevalence of *Pseudomonas aeruginosa* according to the animal product.

Serogroups Distribution of *Pseudomonas aeruginosa*

The distribution diagram showed that the majority of serogroups are from beef. These serogroups are O16, O15, O11, O8, O7, O5, O4 and O1; while, serogroups O12, O9 and O2 were specific to smoked fish. For fresh fish, only O10 serogroups was found. However not serotypeable are both related to beef and smoked fish (Figure 5).

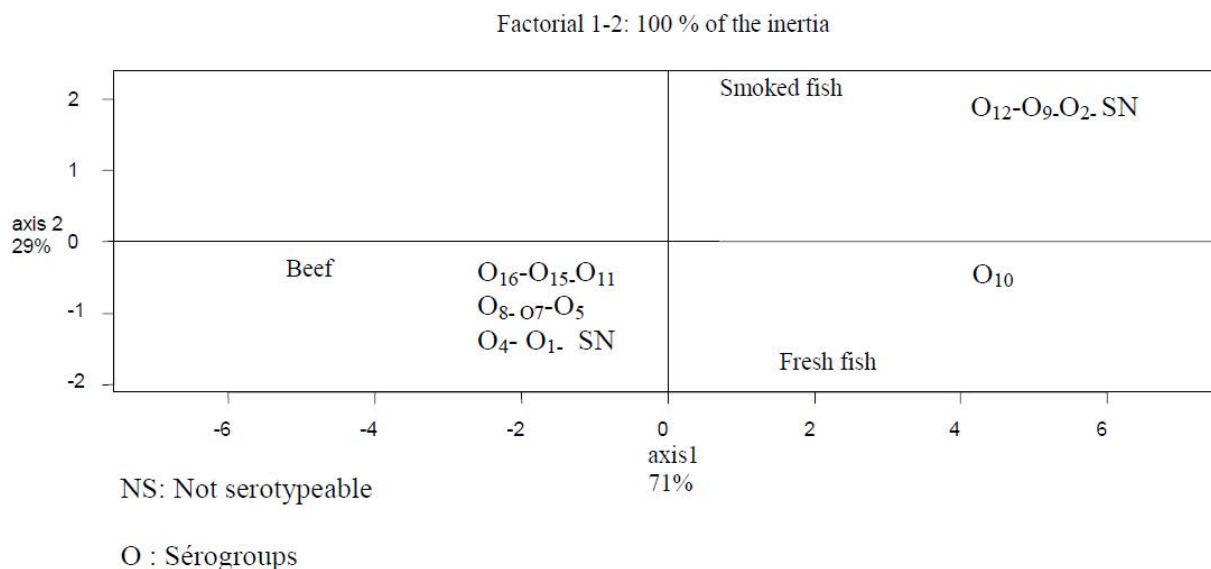


Figure 5. Serogroups distribution of *Pseudomonas aeruginosa* according to the animal product.

DISCUSSION

The identification of *Pseudomonas* following physiological and biochemical characteristics are useful, but it is not enough to distinguish the *Pseudomonas* [3,16]. Also, conventional microbiological methods for identifying of *Pseudomonas* from food, environmental and clinical samples are reliable; they require several days to be completed. Rapid detection of isolates contaminating food is very important for consequent treatment decision for agribusiness [1]. PCR has the potential for identifying microbial species rapidly by amplification of sequences unique to a particular organism [16].

The results of this study confirm that a correct identification and characterization of *Pseudomonas* and *Pseudomonas* species can only be achieved by combining cultural, biochemical and molecular tests. The molecular results showed that 97.5% of presumptive isolates belonged to the *Pseudomonas* genus. This high molecular identification rate showed that genomic studies are needed to confirm the exact taxonomic position of *Pseudomonas* spp^[3,16,28]. The results of the microbiological analysis of animal products showed that the overall prevalence of *Pseudomonas* in animal products was 86.8%. This high prevalence could be justified by the fact that the *Pseudomonas* germ and mainly *P. aeruginosa* is very involved in food spoiling.

This species can adapt to a variety of environments because of its high genetic plasticity. It can also colonize and infect almost all anatomical sites^[2,28,29]. The prevalence of *Pseudomonas* was higher in beef with 97.9%, followed by fresh fish with 87.8%. The lowest prevalence was found in smoked fish with 63.1%. This difference in level of contamination is explained by the fact that *Pseudomonas* develops preferentially in at high water activity products. Indeed, the water activity of beef (72% - 97%) and fresh fish (73% - 95%) are higher than the smoked fish (10% - 30%) and therefore it influences the taste of food and susceptibility to degradation^[18,30].

Furthermore, the high prevalence of *Pseudomonas* observed in beef is similar to that reported by Abd^[1]; Franzetti and Scarpellini^[3]. *Pseudomonas* in the surveyed products showed that beef, fresh and smoked fish sold in markets in Abidjan are impaired because they are indicators of psychrotrophic germs or index of alteration of contaminated products^[3,14,16]. However, the low prevalence of *Pseudomonas* observed in smoked fish could be explained by the fact that during smoking, the smoke particles absorbed by fish, inhibit bacterial growth on the product surface^[30]. The presence of these germs in the smoked fish samples could be explained by post-contamination due to the bad conditions of sale and storage in the markets^[3,31].

A variety of species belonging to the genus *Pseudomonas* was isolated in this study. The majority of *Pseudomonas* species isolated in this study were *P. aeruginosa* (46.8%), *P. putida* (26.6%), and *P. fluorescens* (8.2%). The mainly presence of the three species could be justified by the fact that these species can thrive in various environments as able to grow at ambient temperatures (25-300°C)^[9,30].

These results are similar to those reported by Abd^[1] and Franzetti^[3] who were isolated a variety of *Pseudomonas* species in various food products including beef and fish^[1,3,16]. The high prevalence of *P. aeruginosa* and *P. putida* could be explained by the fact that these two species are commonly found in humans, animals, plants and their involving in zoonosis^[2,9,12]. *Pseudomonas stutzeri*, *P. chlororaphis* and *P. putrefaciens* are lower because they grew in specific conditions of pH, water activity (aw) and temperature^[18].

The prevalence of *P. aeruginosa* and *P. putida* was higher in beef and lower respectively in fresh fish and in smoked fish. This high prevalence in beef could be explained by the fact that this type of product is capable of harboring pathogens such as *P. aeruginosa* species and *P. putida* that are much present in the environment and in the battle of drinking water^[32,33]. This prevalence could also be explained by the fact that these two species are able to live in the mucosa, cavities and body of beef and contaminate the finished product after the slaughter. The prevalence of *Pseudomonas fluorescens*, *P. cepacia*, *P. stutzeri*, *P. putrefaciens* and *P. chlororaphis* was less than 5% in the three types of products. This could be explained by the fact that these species could contaminate least these product types.

The study also highlighted a variety of serogroups of *Pseudomonas aeruginosa* isolated from animal products. The serogroups O11, O5, O7 and O16 are predominantly serogroups isolated in this study. These results could be due to the fact that these serogroups are mostly involved in animal infections^[12]. The same observation was done by Lu et al. who indicated that the serogroups O11 is associated with the production of elastase and some enzymes secreted by the type III secretion system (TTSS) itself involved in pulmonary lesions.

They found in their study that the serogroups O11 strains were the most virulent^[12]. The O11 and O5 serogroups were isolated more in beef than fresh fish and smoked fish. Serogroups O1, O4, O8 and O15 were isolated only in beef; while, serogroups O2 and O10 were isolated respectively in smoked fish and in fresh fish. This diversity of serogroups could be justified by the fact that strains isolated in this study should be in contact with environmental, aquatic and animal strains.

These results agree with those of Lu et al.^[12] who isolated in their study, the same number of *Pseudomonas aeruginosa* serogroups with different prevalences. In contrast to our study, these authors did not isolated O5 and O16 serogroups. However, they isolated the serogroups O3 and O6 with the majority in their study the serogroups O6 (29.3%) and O11 (22.8%). These few differences could be explained by the number of analyzed samples, the nature of the matrices and the sampling of site.

Others studies have shown that the serogroups O1 isolated only in beef and O11 isolated both beef; fresh fish and smoked fish are associated with severe nosocomial infections^[11,12]. These serogroups could be responsible for the secretion of the exoenzyme ExoS, ExoT and ExoU^[2,12]. These enzymes do not initiate infection but promote tissue destruction at the site of inflammation and bacterial dissemination. The ExoS is most often associated with chronic infections due to *P. aeruginosa* by inducing apoptosis^[2,12]. Indeed, when The ExoS enzyme was introduced into the eukaryotic cell, this protein destroyed the actin filaments inducing cytotoxicity and resistance to phagocytosis.

The ExoS and ExoT are related to O11 serogroups are often associated with mortality in animals^[12,14]. The principal

component analysis showed that serogroups O16, O15, O11, O8 O7, O5, O4 and O1 are linked to beef that fresh and smoked fish. The same analysis showed that fresh fish is characterized by serogroups O10 and smoked fish by serogroups O12, O9 and O2. Non serotypeable strains are both related to beef and smoked fish. This belonging serogroups is due to the many virulence factors and the great ability of the microorganism to infect several types of hosts such as plants, insects and animals.

CONCLUSION

The results showed that a correct identification and characterisation of the *Pseudomonas* species can only be achieved by combining physiological, biochemical and molecular tests. The present study has highlighted the presence and diversity of the genus *Pseudomonas* in animal products. The species of *Pseudomonas* mostly isolated were *P. aeruginosa*, *P. putida* and *P. fluorescens*. The study also found a high prevalence in *P. aeruginosa* belonging to serogroups risk. These serogroups of *P. aeruginosa* isolated, the most common and known to be potentially pathogenic were serogroups O11, O5, O7 and O16. All these serogroups could be associated with alteration factors, resistance and virulence of individuals; this requires improving the health management of the food chain of the products analyzed to prevent their possible alteration or reduce the risk of infections that could be related to this case.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Department of Bacteriology and Virology of the Pasteur Institute of Côte d'Ivoire, the Department of Science and Technology of Foods, the Laboratory of Microbiology and Molecular Biology of the University of Nangui Abrogoua as well as producers and sellers meat and fish in Côte d'Ivoire for their contribution in making this work. The authors have not declared any conflict of interests.

REFERENCES

1. Abd El-Aziz DM. Detection of *Pseudomonas spp.* in chicken and fish sold in markets of assiut city, Egypt. J Food Qual Hazards Control. 2015;2:86-89.
2. Bricha S, et al. Virulence factors and epidemiology related to *Pseudomonas aeruginosa*. Tunisian Journal of Infectious Diseases. 2009;2:7-14.
3. Franzetti L and Scarpellini M. Characterization of *Pseudomonas spp* isolated from foods. Ann Microbiol. 2007;57:39-47.
4. Lisboa T, et al. We should be measuring genomic bacterial load and virulence factors. Crit Care Med. 2010;38:656-662.
5. Mitov I, et al. Prevalence of virulence genes among bulgarian nosocomial and cystic fibrosis isolates of *Pseudomonas aeruginosa*. Braz J Microbiol. 2010;41:588-595.
6. Keith P. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. J Mol Microbiol Biotechnol. 2001;3:255-264.
7. Meseret M, et al. Antimicrobial drug resistance and disinfectants susceptibility of *Pseudomonas aeruginosa* isolates from clinical and environmental samples in Jimma University specialized hospital, southwest Ethiopia. American Journal of Biomedical and Life Sciences. 2014;2:40-45.
8. El-Ouali A, et al. Microbiological monitoring of environment surfaces in a hospital in Fez city, Morocco. J Mater Environ Sci. 2016;7:123-130.
9. Iqbal S, et al. Isolation and characterization of Various *Pseudomonas* species from distinct clinical specimens. IOSR Journal of Dental and Medical Sciences. 2015;14:80-84.
10. Nikbin V, et al. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. Iranian Journal of Microbiology 2012;4:118-123.
11. Lu Q, et al. Pharmacokinetics and safety of panobacumab: specific adjunctive immunotherapy in critical patients with nosocomial *Pseudomonas aeruginosa* O11 pneumonia. J Antimicrob Chemother. 2011;66:1110-1116.
12. Lu Q, et al. *Pseudomonas aeruginosa* serotypes in nosocomial pneumonia prevalence and clinical outcomes. Critical Care. 2014;18:R17.
13. Le-Berre R, et al. Relative contribution of three main virulence factors in *Pseudomonas aeruginosa* pneumonia. Crit Care Med. 2011;39:2113-2120.
14. Oku I and Amakoromo ER. Microflora of fresh and smoke-dried fish in Yenagoa metropolis, Nigeria. Afr J Microbiol Res. 2013;7:4451-4456.
15. Golly KJ, et al. Phytochemical study and antimicrobial activity of bark extracts of *Ceiba pentandra* (L.) Gaertn. (Bombacaceae) from Côte d'Ivoire on antibiotic resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Br Microbiol Res. 2015;9:1-7.

16. Virupakshaiah DBM and Hemalata VB. Molecular identification of *Pseudomonas aeruginosa* from food borne isolates. *Int J Curr Microbiol App Sci*. 2016;5:1026-1032.
17. Voloski FLS, et al. Influence of cutting and deboning operations on the microbiological quality and shelf life of buffalo meat. *Meat Science*. 2016;116:207-212.
18. Neumeyer K, et al. Development of a predictive model to describe the effects of temperature and water activity on the growth of spoilage Pseudomonads. *Int J Food Microbiol*. 1997;38:45-54.
19. FAO et al. Livestock sector development for poverty reduction: an economic and policy perspective – Livestock’s many virtues. Roland-Holst, Rome; 2012.
20. FAO et al. The State of World Fisheries and Aquaculture; contributing to food security and nutrition for all. Contributing to food security and nutrition for all, Rome; 2016.
21. Oyase A and Jemerigbe R. Contribution of aquaculture to poverty reduction and food security in Nigeria. *Int J Appl Microbiol Biotechnol Res*. 2016;4:26-31.
22. Wannous DC. The potential contribution of livestock to food and nutrition security: the application of the One Health approach in livestock policy and practice. *Rev Sci Tech Off Int Epiz*. 2014;33:475-485.
23. FAO. United Nations Food and Agriculture Organization. FAO yearbook, Fishery statistics capture production. 2000;86:99-100.
24. Moroh J. et al. Diversity and antibiotic resistance of uropathogenic bacteria from Abidjan. *Afr J Urol*. 2014;20:18-24.
25. http://www.iso.org/iso/catalogue_detail.htm?csnumber=31590
26. http://www.iso.org/iso/catalogue_detail.htm?csnumber=45099
27. http://www.iso.org/iso/catalogue_detail?csnumber=36534
28. Khattab M, et al. Genetic identification of *Pseudomonas aeruginosa* virulence genes among different Isolates. *J Microb Biochem Technol*. 2015;7:274-277.
29. Ramprasad BP, et al. Role of *Pseudomonas* in nosocomial infections and biological characterization of local strains. *Journal of Biosci Tech*. 2010;1:170-179.
30. Idah PA and Nwankwo I. Effects of smoke-drying temperatures and time on physical and nutritional quality parameters of Tilapia (*Oreochromis niloticus*). *International Journal of Fisheries and Aquaculture*. 2013;5:29-34.
31. Lokuruka MN. Food quality perspectives in African fish products: practices, challenges and prospects. *International Journal of Fisheries and Aquaculture Sciences*. 2016;6:15-32.
32. Aman, et al. Prevalence of antimicrobial resistant *Pseudomonas aeruginosa* in fresh water spring contaminated with domestic sewage. *Journal of Biological and Food Science Research*. 2012;1:19-22.
33. Klrissa S and Mohammad K. *Pseudomonas aeruginosa*: A review of their pathogenesis and prevalence in clinical settings and the environment. *Infection, Epidemiology and Medicine*. 2016;2:25-32.