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High Prevalence of Multidrug Resistant *Pseudomonas aeruginosa* Strains Recovered from Bovine Meat, Fresh and Smoked Fish in Côte d'Ivoire

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Abstract

The overuse of antibiotics in animals as a growth-promoting agent and the emergence of bacterial multi-resistance become a concern. The aim of this study was to detect the antimicrobial resistance potential of virulent *P. aeruginosa* isolated from animal products. A total of 153 strains of *P. aeruginosa* multidrug-resistant (PAMDR) were isolated from bovine meat (93), fresh fish (36), and smoked fish (24). API20NE methods, susceptibility testing, serotyping and polymerase chain reaction (PCR) using the 16S gene and the *bla*SHV, *bla*TEM, *bla*IMP, *bla*VIM, *bla*PER, *bla*VEB, *bla*OXA-58 genes were performed. *Pseudomonas aeruginosa* isolates were resistant to aztreonam (83.1%), ticarcillin + clavulanic acid (52.1%), ticarcillin (51.1%) and to ciprofloxacin (42.6%). The Ambler class A, B, and D β-lactamase genes detected were in decreasing importance order, *bla*SHV (28.1%) (p <0.05), *bla*TEM (20.9%), *bla*PER (14.4%), *bla*VEB (13.1%), *bla*IMP (9.8%) and *bla*VIM (6.4%). The prevalence of resistance genes ranged from 0% to 21.5%, from 0% to 27.8%, and from 0% to 54.2%, respectively, in strains of *P. aeruginosa* isolated from beef, fresh fish and smoked fish. The genes studied were emerging resistance determinants in *P. aeruginosa*; representing a risk for consumer as a result of eventual non-compliant animal products. It is necessary to improve the food chain of the products analyzed to avoid the risk of infections linked to *P. aeruginosa*.

Key words: *P. aeruginosa*; multidrug-resistant; bovine meat; fish; β-lactamase genes

Résumé

La surexploitation des antibiotiques chez les animaux en tant qu'agent favorisant la croissance et l'apparition de multirésistance bactérienne deviennent une préoccupation. Le but de cette étude était de détecter le potentiel de résistance antimicrobienne de *P. aeruginosa* virulent isolés de produits animaux. Un total de 153 souches de *P. aeruginosa* multirésistantes (PAMDR) ont été isolées de viande bovine (93), de poisson frais (36) et de poisson fumé (24). Les méthodes API20NE, les tests de sensibilité, le sérotypage et la réaction de polymérisation en chaîne ou Polymerase Chain Reaction (PCR) utilisant le gène 16S, les gènes *bla*SHV, *bla*TEM, *bla*IMP, *bla*VIM, blaPER, *bla*VEB, *bla*OXA-58 ont été réalisées. Les isolats de *P. aeruginosa* étaient résistants à l'aztréonam (83,1%), à la ticarcilline + acide clavulanique (52,1%), à la ticarcilline (51,1%) et à la ciprofloxacine (42,6%). En ordre d'importance décroissante, les gènes de β-lactamase des classes de Ambler A, B et D détectés étaient *bla*SHV (28,1%) (p <0,05), *bla*TEM (20,9%), *bla*PER (14,4%), *bla*VEB (13,1%), *bla*IMP (9,8%) et *bla*VIM (6,4%). La prévalence des gènes de résistance variait de 0% à 21,5%, de 0% à 27,8% et de 0% à 54,2%, respectivement, au niveau des souches de *P. aeruginosa* isolées de viande bovine, de poisson frais et de poisson fumé. Les gènes étudiés sont des déterminants de résistance émergents chez *P. aeruginosa*; ce qui représente un risque pour le consommateur de produits animaux contaminés. Il est nécessaire d'améliorer la chaîne alimentaire des produits analysés afin d'éviter le risque d'infections liées à P. aeruginosa.

Mots clés : *Pseudomonas aeruginosa*; multirésistance; viande bovine; poisson, gène de β-lactamase

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Introduction

Antibiotics represent one of the most important biomedical revolutions of the 20th century and take an important place in human and veterinary medicine (Nathan, 2017). In livestock, a source of food production, they can prevent and treat infectious diseases that can cause significant morbidity and mortality (Abdul et al., 2014). Antibiotics can also be used in the feed as additives to improve animal growth and performance (Dibner and Richards, 2005). According to the French National Institute for Health Surveillance, more than half of all antibiotics produced in the world are administered to animals (Lévi, 2006). However, it is currently recognized that the use of antibiotics in different ecosystems (plants, animals and humans) exerts a selection pressure that promotes the emergence and spread of resistant bacterial strains (Economou and Gousia, 2015; Coetzee et al., 2016). Thus, in response to this pressure, several species isolated in foods developed complex biological mechanisms leading to antibiotic resistances (Economou and Gousia, 2015). This can in turn increase the incidence of human infections due to antimicrobial resistant pathogens (Hao et al., 2014; Tew et al., 2016). Consequently, among animal products, meat and fish, which are the important sources of animal proteins, can serve as sources of multidrug resistant bacteria that can be transferred from food producing animals to consumers (Economou and Gousia, 2015; Tew et al., 2016). Indeed, evidence of resistance genes in food products strains was demonstrated recently (Hao et al., 2014; Tew et al., 2016).

Among the multidrug-resistant bacteria, some opportunistic pathogens such as *P. aeruginosa* can infect virtually any tissue (Virupakshaiah, and Hemalata, 2016). It can infect immunocompromised individuals and responsible for nosocomial infections (Abd, 2015; Khattab et al., 2015). An essential determinant of *P. aeruginosa* strains is their high resistance to certain antimicrobial agents (Meena et al., 2015, Al-Agamy et al., 2016). In addition, Extended-spectrum ß-lactamases (ESBLs), including Pseudomonas Ex-

tended Resistance (PER), Vietnamese Extended spectrum ß-lactamase (VEB), Temoneira-patient name (TEM), Sulfhydryl Variable (SHV), GES and Oxacillinases (OXA) enzymes, are reported in *P. aeruginosa* strains (Pfeifer et al., 2010; Du et al., 2010; Al-Agamy et al., 2016). Also, different types of MβL such as Imipenemase (IMP), Verona imipenemase (VIM) and German imipenemase (GIM), have been identified among *P. aeruginosa* strains (Vahdani et al., 2012; Khorvash et al., 2015; AL-Kadhmi et al., 2016). Thus, Infections caused by *P. aeruginosa* are therefore most often treated by carbapenems as medicines of last resort (Al-Agamy et al 2016). *However*, isolation of *P. aeruginosa* strains of various origin resistant to carbapenem is concern and urgent because this resistance is often linked to resistance genes (Mohanty et al., 2013; Sibhghatulla et al., 2015; Tew et al., 2016). Recently, potential virulent *P. aeruginosa* strains were detected from bovine meat, fresh and smocked fish as widely consumed in Côte d'Ivoire (Benie et al., 2017a). In this paper, the antimicrobial resistance potential of these *P. aeruginosa* strains was evaluated.

Materiels and Methods

Isolation and identification of *P. aeruginosa*

Pseudomonas aeruginosa was isolated from bovine meat, fresh fish and smocked fish by using selective medium (Pseudomonas Cetrimide Agar) incubated at 37ºC for 24 hours. The isolates were identified based on the morphological and biochemical characters by API 20NE (bioMérieux, Marcy l'Etoile, France). For molecular identification, bacterial total DNA was extracted using a fast-boiling method (Kor et al., 2013; Mitov et al., 2010). Plasmid DNA was extracted using the DNA-spinTM Plasmid DNA Purification Kit (Intron, South Korea). The purity and DNA concentration of the extracts were determined by spectrophotometer (Eppendorf BioPhotometer plus, USA) Eppendorf B (2019).

PCR mixtures with a final volume of 25 µl consisted of 16 µl sterile Milli-Q water (Milli-Q™, Millipore Corporation, USA), 5 µl 5XTP, 1.5 µl MgCl2 (2 mM), 0.2 µl dNTPs (10 mM), 0.1 µl each primer (20 mM) (Integral DNA Technology, France), 0.1 µl Go tag polymerase (Promega Corporation, Madison, WI 53711-5399, USA), and 2 µl template DNA. Pseudomonas aeruginosa were identified by 16S gene (Table 1). The reference strain *P. aeruginosa* PA 14 was used as quality control. The PCR products were purified using a commercial kit (EZ-10Spin Column PCR Product Purification Kit, Canada) and sequenced with primer 27F and 1492R in automated 310 DNA sequencer (Applied Biosystem, Foster City, CA). The resulting sequences were analyzed using BLAST in the NCBI database (www.ncbi.nlm.nih.gov) for strain characterization.

Antimicrobial drugs susceptibility testing

Antimicrobial susceptibility testing of *P. aeruginosa* isolates was performed by diffusion method on Mueller-Hinton agar (MHA; Bio-Rad, Marnes-La-Coquette, France). The antibiotics tested and their sensidisk concentrations were ticarcillin (TIC; 75 µg), ticarcillin-clavulanic acid (TCC; 75-10 µg), aztreonam (ATM; 30 µg), cefepime (FEP; 30 µg), ceftazidine (CAZ; 10 µg), ciprofloxacin (CIP; 100µg), colistin (CST; 10 µg), imipenem (IPM; 10 µg), piperacillin (PIP; 100µg), fosfomycin (FOS; 200µg) and kanamycin (K; 30 µg). Accordingly, 5 selected colonies of *P. aeruginosa* were taken from a pure culture and transferred to a tube containing 5 ml sterile nutrient broth and mixed gently until a homogenous suspension was formed (NF EN ISO 6887, 2004).

Isolated bacteria was grown in nutrient broth and incubated for 4-6 hours at 37°C until the turbidity was matched with the 0.5 McFarland standards. The bacteria suspension adjusted to McFarland turbidity was evenly swabbed over the entire surface of Mueller Hinton agar (Bio-Rad, Marnes-la-Coquette, France) (Oxoid, England) using sterile cotton swab. The inoculated plates were then incubated at 37°C for 24 hours.

Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler, which was held on the back of the inverted Petri plate, and the isolates were classified as sensitive, intermediate and resistant according to the standardized table recommended by the CA-SFM/EUCAST (European Committee on Antimicrobial Susceptibility Testing) (CA-SFM/EUCAST, 2015). The standard reference strain of *P. aeruginosa* ATCC 27853 was used as a quality control for culture and in each of antimicrobial susceptibility testing throughout the study.

Serotyping of 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, France), according to the manufacturer's recommendations.

Phenotypic identification of metallo-β-lactamases (MBL)-producing isolates

All strains that showed reduced susceptibility to imipenem ≥8 g/ml were screened for MBL production. These strains were subjected to a phenotypic analysis by EDTA (Sigma Chemicals, St. Louis, MO) combination disk test (Deeba et al., 2011). Briefly, a 18hours culture of animal isolate was diluted with peptone water (Oxoid, USA) corresponding to the 0.5 McFarland standard, which is approximately 108CFU/ml and spread on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England) plate using cotton swab.

Two IPM (10µg) disks were placed on the surface of the agar at distances of 20 mm away from each other. Then, 4µL of EDTA (0.5 M, pH 8) solution was added to one of the IPM (10µg) disks. Another IPM disk (10 µg) was placed at 20 mm center to center of a sterile non-impregnated disk on which 10µl of EDTA (0.5 M, pH 8) has been added. The inhibition zones displayed around the IPM and the IPM-EDTA disks were compared after 18 to 24hrs incubation at 37ºC. The difference of ≥ 7mm between the inhibition zone diameter of the IPM-EDTA disk and that of the IPM (10µg) alone disk was a positive test for the presence of MBLs.

Detection of ß-Lactamase (bla) genes by polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) amplification of genes for Ambler classes A, B, and D beta-lactamase enzymes was performed using specific primers (blaVEB, blaPER, blaTEM, blaSHV, blaIMP, blaVIM, and blaOXA-58) (Table 2). Multiplex PCRs for characterization of blaVEB, blaPER, blaTEM, blaSHV, blaIMP, blaVIM, and blaOXA-58 genes were carried out with 25µL reaction mixture consisting of 15.8µL of sterile Milli-Q water (milli-Q ™, Millipore Corporation, USA), 5µL of 5XTP, 1.5µL of MgCl2 (2 mM), 0.2 µL of dNTPs (10 mM), 0.1µL of each primer (10 mM) (Integral DNA Technology, France), 0.1µL of Go tag polymerase (Promega Corporation, Madison, WI 53711-5399, USA) and 2µL of the DNA template. A negative control containing 2µL of sterile Milli-Q water in place of DNA template and DNA of the ATCC reference strain 27853 used for positive control was included in each PCR reaction. All amplification reactions were carried out in a thermocycler of type T3000 Thermocycler, Block type standard 3a, (Biometra, Germany). The amplification products (10µL) of resistance genes were separated by gel electrophesis on 2% agarose gel containing 0.5 µg/ml ethidium bromide

Table 1 : Primers used for molecular identification genes in single PCR

Target gene	Primers	sequence (5'-3')	Amplification Program	Product size (bp)	Annealing temperatue (°C)	References
16S	27F	AGAGTTTGATCMTGGCTCAG	94°C, 5 min 35 x [94°C, 30s; 55°C, 40s; 72°C, 1 min 30s] 72°C, 10min; 4°C …	1500	55	(Amutha et Kokila, 2014)
	1492R	TACGGYTACCTTGTTACGACTT				

for 35 min at 120 V. A volume of 7 µl of a molecular weight marker (TriDyeTM, 100 bp or 1kb DNA Ladder, Biolabs) was included in all gels to estimate the size of the DNA bands. The gels were visualized by Molecular Imager Gel DocTM EZ (Bio-Rad, USA). Amplified genes were identified based on fragment size shown in Table 1 and Table 2.

Statistical analysis

The statistical analysis was carried out on the software Statistical Package for the Social Sciences (SPSS) 20.0 (IBM SPSS, Chicago, IL, United States of America) using the Student's t test, Mann-Whitney U test, Spearman's correlation analysis and multiple regression analysis. Statistical significance was set at $p < 0.05$.

Table 2 : Primers used for amplification of resistance genes in multiplex PCR. Extended-spectrum ß-lactamases (ESBL), including Pseudomonas Extended Resistance (PER), Vietnamese Extended spectrum ß-lactamase (VEB), Temoneira-patient name (TEM), Sulfhydryl variable (SHV), (oxacillinases) OXA enzymes and MβL Imipenemase (IMP), Verona imipenemase

Target Genes	Primers	sequence (5'-3')	Amplification Program	Product size (bp)	Annealing temperature (°C)	References
bla PER	PER-F	GTAGTATCAGCCCAATCCCC		738	60	(Melano et al., 2003)
	PER-R	CCAATAAAGGCCGTCCATCA	94°C, 5 min			
bla TEM	TEM-F	ATGAGTATTCAACATTTCCGTG	35 x [94°C, 35 s ; 60°C, 1min ;	840	60	(Kruger et al., 2004)
	TEM-R	TTACCAATGCTTAATCAGTGAG	72°C,1min]			
bla SHV	*SHV*-F	TTTATGGCGTTACCTTTGACC	72°C, 7 min ; 4°C …	1,051	60	(Yagi et al., 2000)
	SHV-R	ATTTGTCGCTTCTTTACTCGC				
bla VIM	*VIM*-F	TGGTCTACATGACCGCGTCT		766	60	(Touati et al., 2013)
	VIM-R	CGACTGAGCGATTTGTGTG				
bla IMP	*IMP*-F	CATACTCGTT-GAAGAAGTTAACGG	94°C, 5 min 35 x [94°C, 35s ; 63°C, 1min; 72°C, 1 min] 72°C, 7 min ; 4°C …	448	63	(Kulah et al., 2010)
	IMP-R	GAGAATTAAGCCACTCTATTGC				
bla VEB	*VEB*-F	GGAACAACTTTGACGATTGA	94°C, 5 min 35 x [94°C, 35s ; 56°C, 1min; 72°C, 1min] 72°C, 7 min ; 4°C …	374	56	(Melano et al., 2003)
	VEB -R	CCCTGTTTTATGAGCAACAA				
OXA-58	OXA-58A	CGATCAGAATGTTCAAGCGC	94°C, 5 min 35 x [94°C, 35s ; 61°C, 1min; 72°C, 1min] 72°C, 7 min ; 4°C …	529	61	(Martínez et al., 2009)
	OXA-58B	ACGATTCTCCCCTCTGCGC				

Results

Antimicrobial susceptibility of the isolated *P. aeruginosa* strains

The isolated strains were confirmed to be *P. aeruginosa* species by both API 20NE and 16S rDNA sequence analyses (Fig. 1, Table 3). The prevalence of *P. aeruginosa* found in animal product is higher in bovine meat (93: 60.8%) than fresh fish (36: 23.5%) and smoked fish (24 : 15.7%) (Table 4). The percentages of *P. aeruginosa* isolates resistance were 83.1% for aztreonam, 52.1% ticarcillin, 51.1% ticarcillin+clavulanic acid, 42.6% ciprofloxacin, 39.2% piperacillin, 27.1% cefepime, 22.8% ceftazidim, 8.3% imipenem, 7.1% colistin and 0% fosfomycin (Fig. 2). Moreover, eight (O1; O5; O7; O9; O11; O12; O15 and O16) serogroups were identified among the 153 isolates with factors O11, O5 and O1 being predominant (Table 5); the isolated *P. aeruginosa* strains were identified as MBL-producing strains (Fig. 3).

Prevalence and distribution of resistance genes

The blaSHV gene was the most detected with 28.1% followed by blaTEM (20.9%) (Table 5).
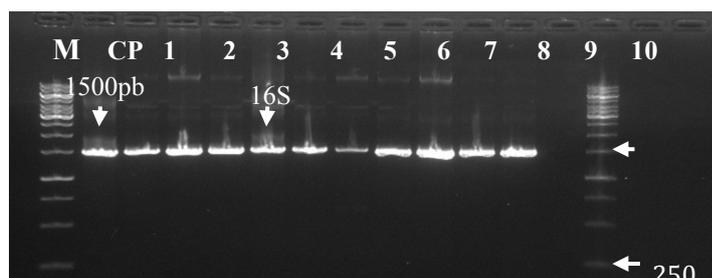The prevalence of blaPER and blaVEB was 14.4% and 13.1%, respectively.

Figure 1 : 16S profiles of *P. aeruginosa* isolates. Lanes 1-10 : Presence of *P. aeruginosa* in animal products; PA14: Positive control (*P. aeruginosa* PA 14); C-: Negative control; M: Marker Gene Ruler 250 bp (Bench Top, 1kb DNA Ladder, Promega Corporation, USA).
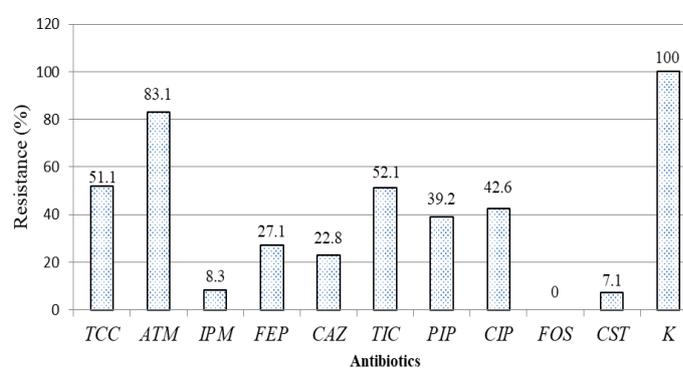

Figure 2 : Antimicrobial resistance in strains of *P. aeruginosa* isolated from animal products.
Ticarcillin (TIC), ticarcillin-clavulanic acid (TCC), aztreonam (ATM), cefepime (FEP), ceftazidine (CAZ), ciprofloxacin (CIP), colistin (CST), imipenem (IPM), piperacillin (PIP), fosfomycin (FOS) and kanamycin (K).

Table 3: Confirmation of the bacterial strains as *P. aeruginosa* species by the 16S rDNA sequence analyses

Isolates	Blast hits	Homology %
DB31	*Pseudomonas aeruginosa* (JQ773433)	99.7
DB43	*Pseudomonas aeruginosa* (MF289196)	99.7
DB71	*Pseudomonas aeruginosa* (KT005274)	99.8
DB98	*Pseudomonas aeruginosa* (MF356919)	99.7
DB103	*Pseudomonas aeruginosa* (MF356919)	99.6
DB111	*Pseudomonas aeruginosa* (MF356919)	99.0
DB 112	*Pseudomonas aeruginosa* (MF797204)	99.9
DB 113	*Pseudomonas aeruginosa* (KT946130)	99.7
DB119	*Pseudomonas aeruginosa* (KX149022)	99.6

The resulting sequences were analyzed using BLAST in the NCBI database (www.ncbi.nlm.nih.gov) for strain characterization.

This prevalence was less than 10% for the blaIMP and blaVIM genes. The blaOXA-58 gene was not detected in this study. The prevalence of resistance genes ranged from 0% to 21.5%, from 0% to 27.8%, and from 0% to 54.2%, respectively, in strains of *P. aeruginosa* isolated from beef, fresh fish and smoked fish (Fig. 4). The three matrices as well as SHV and TEM were positively correlated to axis 1. IMP, OXA-58 and VIM were negatively correlated to axis 1(Fig. 5). It is infered that the three meat products contain large quantities of SHV and TEM but IMP and VIM were found in small quantities (Fig. 5). However, the matrices are different from each other in relation to the number of genes found.
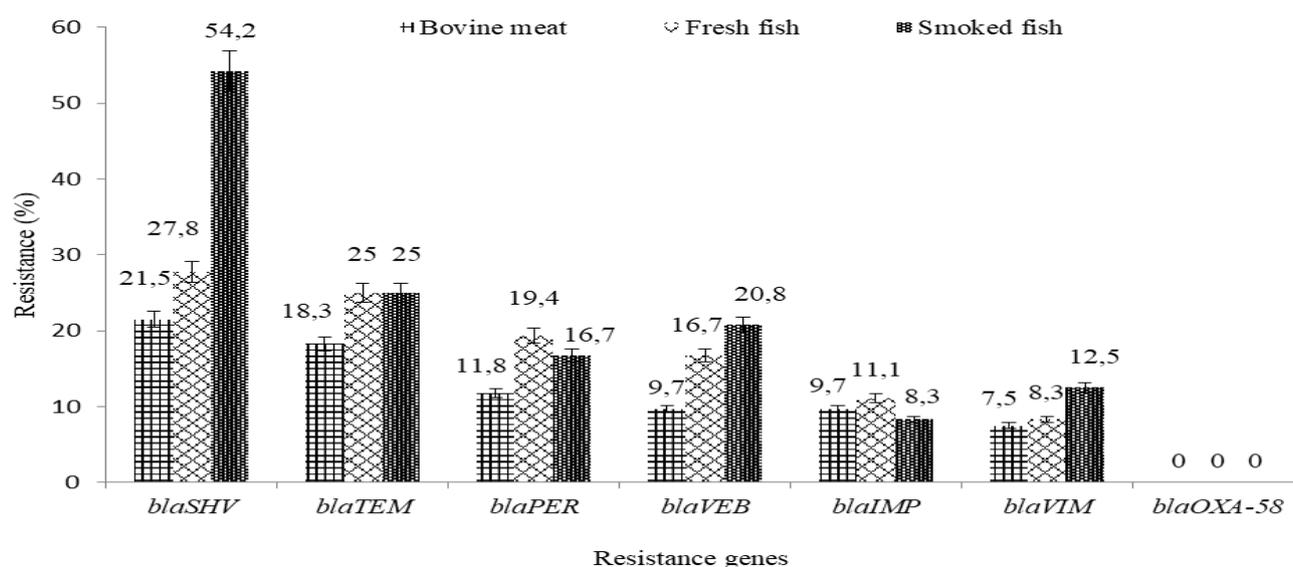

Figure 3 : Detection of *P. aeruginosa* producing metallo-ßlactamase. *P. aeruginosa* isolated from animal product (A); positive Hodges test for animal isolates (B). The use of both the Hodges test and an IPM-EDTA improves the detection of metallo-β-lactamase. Imipenem (IPM); 1: IMP disks (10μg) + 4 μlEDTA (0.5 M, pH 8); 2: IMP disks (10μg) without EDTA (0.5 M, pH 8); 3: non-impregnated disk+ 10 μlEDTA (0.5 M, pH 8); 4: IMP disks (10μg) without EDTA (0.5 M, pH 8).

Figure 4 : Resistance of resistance genes according to the origin of the strain. Extended-spectrum ß-lactamases ; (ESBLs), including Pseudomonas Extended Resistance (PER), Vietnamese Extended spectrum ß-lactamase (VEB), Temoneira-patient name (TEM), Sulfhydryl variable (SHV), (oxacillinases) OXA enzymes and MβL Imipenemase (IMP), Verona imipenemase (VIM).

Discussion

Bovine meat, fresh and smocked fish are widely consumed in Côte d'Ivoire. It means that the safety of these products is a public health concern. Recently, potentially virulent *P. aeruginosa* strains were isolated from bovine meat, fr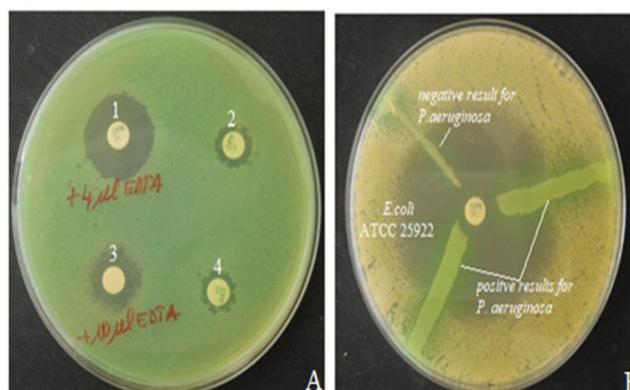esh and smocked fish in Côte d'Ivoire (Benie et al., 2017a). The virulence of a microorganism is not related to drug resistance gene but rather to virulence factors (Benie et al., 2017a ; Benie et al., 2017b).This study was the first on *P. aeruginosa* prevalence and profiles within bovine meat, fresh and smocked fish in Côte d'Ivoire.

Table 4 : Prevalence of *P. aeruginosa* resistance genes. Extended-spectrum ß-lactamases (ESBL), including Pseudomonas Extended Resistance (PER), Vietnamese Extended spectrum ß-lactamase (VEB), Temoneira-patient name (TEM), Sulfhydryl variable (SHV), (oxacillinases) OXA enzymes and MβL Imipenemase (IMP), Verona imipenemase (VIM).

Resistance genes *(ESBL)*	Prevalence of *P. aeruginosa* virulence genes							
	Bovine meat **(n=93)**		Fresh fish (n=36)		Smoked fish (n=24)		Total (n=153)	
	Effective (N)	Prevalence (%)	Effective (N)	Prevalence (%)	Effective (N)	Prevalence (%)	Effective (N)	Prevalence (%)
bla_{SHV}	20	21.5	10	27.8	13	54.2	43	28.1
bla_{TEM}	17	18.3	9	25.0	6	25.0	32	20.9
bla_{PER}	11	11.8	7	19.4	4	16.7	22	14.4
bla_{VEB}	9	9.7	6	16.7	5	20.8	20	13.1
bla_{IMP}	9	9.7	4	11.1	2	8.3	15	9.8
bla_{VIM}	7	7.5	3	8.3	3	12.5	13	6.4
bla_{OXA-58}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5 : Resistance and serogroups of *P. aeruginosa* isolated. Extended-spectrum ß lactamases (ESBL), including Pseudomonas Extended Resistance (PER), Vietnamese Extended spectrum ß-lactamase (VEB), Temoneira-patient name (TEM), Sulfhydryl variable (SHV), (oxacillinases) OXA enzymes and MβL Imipenemase (IMP), Verona imipenemase (VIM). NS: Not Serotypeable, O: Serogroups.

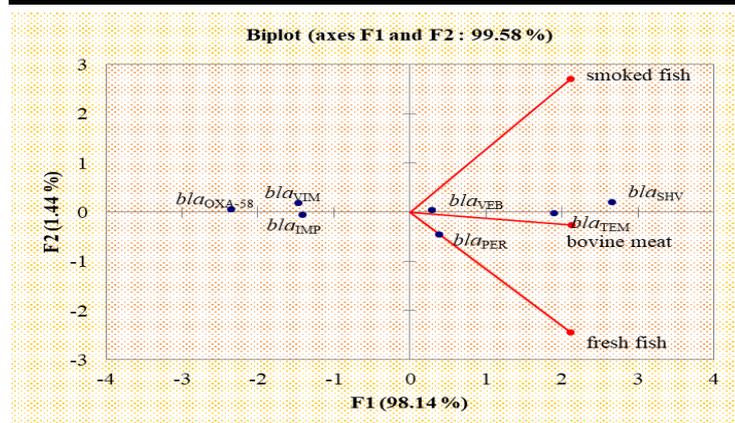| Resistance genes	Serogroups of *P. aeruginosa* isolated (N=153)								
	NS	O_1	O_{11}	O_{12}	O_{15}	O_{16}	O_5	O_7	O_9
bla_{SHV}	15	5	28	3	4	2	15	3	0
bla_{TEM}	12	6	25	2	1	5	8	2	3
bla_{PER}	6	6	12	0	0	1	12	4	1
bla_{VEB}	10	5	10	0	0	3	4	4	2
bla_{IMP}	3	2	5	0	1	1	2	0	1
bla_{VIM}	2	3	4	0	0	2	2	0	0
bla_{OXA-58}	0	0	0	0	0	0	0	0	0

Figure 5 : Distribution of *P. aeruginosa* resistance genes isolated from animal products. Extended-spectrum ß-lactamases (ESBLs), including Pseudomonas Extended Resistance (PER), Vietnamese Extended spectrum ß-lactamase (VEB), Temoneira-patient name (TEM), Sulfhydryl variable (SHV), (oxacillinases) OXA enzymes and MβL Imipenemase (IMP), Verona imipenemase (VIM).

P. aeruginosa prevalence in the current study is similar to other studies carried out on burns and non burns patients by Sheikh et al.(2014) in Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran. In their study, blaIMP and blaVIM genes were detected in 11.7% and 0.4% respectively. In other studies, the prevalence of Ambler class A, B, and D β-lactamase genes detected in *P. aeruginosa* isolated from canine and human infections at Institute of Veterinary Medicine, National Taiwan University, Taiwan, ROC ranged from 8.7% (blaSHV-1) to 100% (blaTEM-1) (Du et al., 2010). Indeed, the results of this study is confirmed by the values obtained by Du et al. (2010) who isolated strains of *P. aeruginosa* producing BLSE with higher prevalence. These over-referred results and those obtained by Mohammad et al., (2016) who have shown multi-resistant strains of *P. aeruginosa* from clinical specimens also confirmed our results in this study.

This multi-resistance could be due to the production of hydrolytic enzymes and the acquisition of resistance mechanisms by strains of *P. aeruginosa* (Rostamzadeh et al., 2016; Syed et al., 2016). Thus, the difference between prevalence in meat and fish could be explained by different use of antibiotics in two production systems. Antibiotics are mostly used in livestock than fish farm. The multi-resistance is higher in fresh products (Bovine meat and Fresh fish) than in smoked product (Smoked fish).

The high prevalence of multi-resistant strains of *P. aeruginosa* in fresh products may be explained by the fact that *P. aeruginosa* colonizes more wet sites (Ibrahim et al., 2015; Al-Zaidi, 2016). This high prevalence could also be justified by the destructive effect of heat on certain antibiotics as indicated by several authors such as Roca et al., (2011); Salah et al., (2013).

The analyzes of results following genes showed that, the blaSHV gene with 49.0% was the most detected followed by blaTEM with 41.8% and this could be explained by the fact that this enzyme (SHV) determines a high level of resistance to ceftazidim and monobactams (Strateva and Yordanov, 2009 ; Du et al., 2010). Therefore, the resistance of *P. aeruginosa* strains isolated from bovine meat, fresh and smoked fish observed in monobactam (Aztreonam), penicillin (piperacillin, ticarcillin) and ceftazidim could be due to the blaTEM and blaSHV gene. The high prevalence of these blaTEM and blaSHV genes can also be explained by the fact that most ESBLs are members of the TEM and SHV-lactamase families. This presence of blaTEM and blaSHV gene would be justified by the numerous mutations that take place leading to a diversity of TEM (> 130) and SHV (> 50) (De Champs et al., 2004). The majority of ESBL are due to genetic mutations of natural beta-lactamases, TEM-1, TEM-2 and SHV-1, active against penicillin's, less against first-generation cephalosporins. The prevalence of blaPER and blaVEB was 27.5% and 24.8% respectively. This indicates that these blaPER and blaVEB genes are responsible for the resistance to ceftazidime observed in the strains of *P. aeruginosa* isolated from animal products. Some epidemiological studies have shown that up to 40% and 80% of *P. aeruginosa* strains resistant to ceftazidime produced VEB-1 (Naas et al., 2008). These same observations were made by some authors who showed that the enzyme VEB-1 (38% identity with PER-1) was found in 1996 in an *E. coli* strain isolated from a Vietnamese patient and then from *P. aeruginosa* in Thailand (Naas et al., 2008). The prevalence of the blaIMP and blaVIM genes (10%) confirms the resistance to carbapenems (imipenem) observed in *Pseudomonas aeruginosa* strains isolated from animal products; Therefore, these enzymes could indicate hydrolytic activity against carbapenems. These results agree with those obtained recently by de Khorvash et al. (2015),

who detected blaVIM genes in 7 (14.6%) strains *P. aeruginosa*. The blaOXA-58 gene was not detected in this study and therefore these strains of animal origin would not harbor genes coding for oxacillinases. In addition, the results of this study revealed that strains of *P. aeruginosa* with resistance factors were mainly strains of serogroups O11, O5 and O1. Therefore, these strains may be involved in various animal and human infections. Principal component analysis also indicated that the three matrices contain the blaSHV and blaTEM gene in large quantities than blaIMP and blaVIM. However, the matrices are different from each other in relation to the number of genes found. These results could be due to the irrational use of antibiotics in the treatment of animal products. ESBL (large-spectrum β-lactamases) and mainly MBL (metallo-β-lactamases) have a major therapeutic impact in *P. aeruginosa* (Khorvash et al., 2015). In the last decade, many class A, B and D β-lactamases have been detected in strains of *P. aeruginosa* (Zhao and Hu, 2010).

Conclusion

The study showed multi-resistance among strains of *P. aeruginosa* of animal origin. It also revealed that strains of *P. aeruginosa* isolated from animal products carry some distinct resistance genes. The blaSHV and blaTEM genes are the most detected in the three food matrices. The study also revealed that strains of serogroups O1, O5 and O11 were more associated with the resistance genes of *P. aeruginosa* isolated from animal products.

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