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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(1): 135-138

www.thepharmajournal.com
Received: 16-11-2019
Accepted: 20-12-2019

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Profile of resistance and serotypage of *Pseudomonas aeruginosa* isolated of African giant's snails

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Abstract
Pseudomonas aeruginosa was sought by feet swabbing of 201 specimens belonging to the 3 African giant snail species present in Ivory Cost (*Achatina fulica*, *Achatina achatina* and *Archachatina ventricosa*). These snails were collected on the markets, and environment. The sensitivity to antibiotics Antibiotic susceptibility of isolated bacteria was achieved by the standard method of diffusion in agar media. Then the serotypage of these strains was carried out by the technique of agglutination on slide using specific polyvalent and monovalent antiserums, according to the manufacturer's protocol (Bio-rad®).
The aim of this study was to investigate the antibiotic resistance profile and serotypes of colonizing snails of Pseudomonas aeruginosa in order to use this bacterium as a biomarker used as an indicator of the medical quality of these snails. The results show resistance rates of 52.7% for tircacillin, 13.8% for tircacillin + clavulanic acid, 05% for cesfulodin and 0% for piperacillin, imipenem and ceftazidim. These rates are based on collection sites and snail species. After serotypage these strains, five serotypes were obtained: O4 (30.4%); O1 (26%); O6 (17.3%); O11 (13.4%); O15 (13.4%). Diversity was also observed in the distribution of serotypes according to collection media and snail species as well as in the resistance of these serotypes to different antibiotic molecules.

Keywords: Snails, *Pseudomonas aeruginosa*, resistance, serotypes

Introduction
In Côte d'Ivoire, African giant snails have a very high food value for the population. They are also consumed in rural areas rather than urban areas and sold in several ways (fresh, smoked, braised). Their flesh contains many amino acids and minerals. It is an important alternative source of animal protein and contributes to the food safety of populations. However, these gastropods, with their lifespan (wetland habitat, herbivore), can be colonized by environmental bacteria and could constitute a danger to public health. This is the case of *Achatina fulica* which has been identified as the intermediate host in two cases of human encephalitis (angiostrongyliosis) caused by *Angiostrongylus cantonensis* [1]. Similarly, some bacteria potentially pathogenic to humans, such as Salmonella sp, have been identified on *Helix aspegia* species [2]. However, the antibiotic resistance profile of these bacteria is unknown as well as the presence of possible virulence genes that may favour certain nuisances if ingested. It therefore seems appropriate to control the bacterial ecology of snail species consumed in Côte d'Ivoire in order to assess possible health risks. Therefore, the search for bacteria that indicate the sanitary quality of these giant African snails offers interesting prospects. This is why the choice is oriented towards *Pseudomonas aeruginosas* because this environmental bacterium shares the same habitat as snails. She has a strong ability to adapt to a hostile environment. It has many virulence and antibiotic resistance genes that it can transmit to snails. *P. aeruginosa* may be involved in foodborne outbreaks [3]. It is highly pathogenic for weakened or immune compromised subjects [4]. Finally, this bacterium is implicated in hospital epidemics due to different serotypes, including O11, which is of environmental origin [4]. It is therefore important to know the serotypes and antibiotic sensibility of strains of *Pseudomonas aeruginosa* that colonize African giant snails in Côte d'Ivoire in order to assess the health risk associated with the consumption and/or handling of these snails.

Material and Methods
Types and sites of study
It concerned a descriptive study realized for two months (from February 2012 to Mars 2012).

The snails were collected from three main sites in the municipality of Yopougon, north of the city of Abidjan. Site A is an urban environment dominated by brush and household and human waste. Site B is a peri-urban environment. This site is characterized by vegetation variability. It is also surrounded by agro-pastoral activities. Site C is composed of the markets of this municipality of Yopougon. This site is characterized by a significant mixing of human populations and various commodities.

Specimens of study

This study concerned African giant snails, *Achatina achtina*, *Achatina fulica* and *Archachatina ventricosa*. All snails belonging to the above-mentioned species were included in this study. Snails were collected on the markets for the species A. achatina and some specimens of *A. ventricosa*. *Achatina fulica* and other species were collected in their natural habitat. For snails collected in their natural area, each specimen was collected with sterile gloves and transported in each bag in a bin for the laboratory. On the market, a batch of five snails was purchased with each saleswoman present during our visit. These snails were also transported to the laboratory in a tank. The identification of snail species was carried out on the basis of phonotypic characteristics.

Bacteriological methods

The bacteria were searched on the feet of different snails. For each sample, a foot smear was performed using a sterile cotton swab moistened with sterile distilled water. These swabs were cultured on cetrimid agar and incubated at 37 °C in 24 to 48 hours. Bacteria identification was carried out using bacteriological technical standards. These were morphological (negative bacillus gram, polar mobility), biochemical (cytochrome oxidase, ß galactosidase), and cultural (increase on cetrimid agar, pigment production in king A and king B agar).

Sensibility to the antibiotics

The Sensibility of strains to antibiotics was studied by a method of diffusion in agar medium according to the recommendations of the Antibiotics Committee of the French Society of Microbiology (CA-FSM). The antibiotics tested were piperacillin 75g, cesfulodin 30g, ticarcillin 75g, ticarcillin + clavulanic acid 85g/10µg, ceftazidime 30µg and imipenem 10g. A quality control was carried out with P. aeruginosa ATTC 27853.

Serotypage

The serotypage of these bacteria was determined by technical agglutination on slide with specific monovalent and polyvalent antisera according to the bio-rad protocol. This method makes it possible to determine 16 serological groups based on thermostable polysaccharide O antigens.

Data analysis

The data have been collected on survey sheet. The variables studied were the species snails, the collect sites, the sensibility profile to the antibiotics of bacterias isolated and different serotypes. These data have been analyzed by software OpenEpi (CDC) 3.01.

Results

Number of specimen collected

The study included 201 snail specimens collected, namely *A. achtina* (25), *A. fulica* (156) and *A. ventricosa* (20).

Bacteriological results

Of these 201 snails collected, 38 strains of *Pseudomas aeruginosa* were isolated. The distribution of isolated bacrterias by collection site (Table I) shows that *P. aeruginosa* was more isolated in urban areas. Depending on the snail species, this table shows that this bacterium was more present in *A. fulica* than in other species (Table I).

Resistance to the antibiotics

Among the collected strains, 36 of them approximately 95% have engendered a study of the sensibility to the antibiotics. The study of sensibility of strains of *P. aeruginosa* has showed that the resistance rate more high has been observed with the tircacillin (52,7%) and any resistance hasn't been observed for the majority of molecules (Table 1). This resistance profile on the three sites has showed a high rate for this same tircacillin molecule. This rate was of 40% on the market, 49% in urban are and 46,7% for the environment periurban. Any resistance has not also been observed for the piperacillin, Imipenem, cefsulodin and the ceftazidim on the all collect sites. Concerning the rate got for the cefsulodin, it was the more high on the market (20%) than on the others sites. According the snails species, the resistance to the tircacillin is only observed for the strains collected on *A. achatina* (50%) and on *A. fulica* (57%) (Table1).

Table 1: Resistance to antibiotics of *P. aeruginosa*

		% of the Resistance					
		TIC	**TCC**	**PIP**	**IPM**	**CFS**	**CAZ**
		52,7	**13,8**	**0,0**	**0,0**	**5,0**	**0,0**
Sites	Market	40,0	0,0	0,0	0,0	20,0	0,0
	urbain	49,9	17,4	0,0	0,0	0,0	0,0
	Periurbain	46,7	12,5	0,0	0,0	4,3	0,0
Species of snails	*A. achatina*	50,0	0,0	0,0	0,0	25,0	0,0
	A. ventricosa	0,0	0,0	0,0	0,0	0,0	0,0
	A. fulica	57,0	15,5	0,0	0,0	3,3	0,0

TIC: ticarcilli; TCC: ticarcillin + clavulanic acid; PIP: piperacillin; IPM: Imipenem; CFS: cefsulodin; CAZ: ceftazidim

Serotypage

A total of 36 strains have created a serotype.74% were typables, 26% were not serotypales. The principal five serotypes were: O4(30,4%); O1(26%); O6(17,3%); O11(13,4%); O15(13,%) (Table II). The prevailing serotypes were O1 and O4. The others serotypes O2; O3; O5; O7; O8; O9; O10; O12; O13; O14; O16 were not isolated. The dividing of these serotypes of *Pseudomonas aeruginosa* in the collect sites was presented as follows: O1 (66, 6) was more represented in urban area than on the others sites, contrariwise O4 (14, 2) is less represented on the market (Table 2). O6 was not detected in the periurban environment. O11 was solely isolated in the periurban environment (Table 2). According the species of snails, the serotype O1 was presented in three species snails with a rate of (66, 6) more high in *A. fulica*. In this same specie the serotype O4 is again more represented with a rate (85, 7%). The O11 was solely isolated in *A. fulica*. Concerning the O15, it occurs in two species excepted *A. ventricosa* (table 2). Concerning the resistance profile of these serotypes *Pseudomonas aeruginosa*, the tircacillin presented a resistance rate of 50% for the O1; 71, 4% for the O4; 66, 6% for the O11 and 33, 3% for the O15.These rates are the highest for all molecules tested. For the molecules tircacillin + clavulanic acid, the high rates have been obtained for the

serotypes O11; O15 (33, 3%) (Table 3).

Table 2: Differents serotypes of *P. aeruginosa*

		n (%) of different serotypes				
		O :1	O :4	O :6	O :11	O :15
		6(26,0)	7(30,4)	4(17,3)	3(13,4)	3(13,4)
Sites	Market	2(33,3)	1(14,2)	1(25,0)	-	1(33,3)
	urban	4(66,6)	3(42,8)	3(75,0)	3(100,0)	1(33,3)
	Periurban	-	3(42,8)	-	-	1(33,3)
Species of snails	*A. achatina*	1(16,6)	1(14,2)	1(25,0)	-	1(33,3)
	A. ventricosa	1(16,6)	-	3(75,0)	-	-
	A. fulica	4(66,6)	7(85,7)	-	3(100,0)	2(66,6)

Table 3: Resistance to antibiotics of the serotypes

		% of the resistance of the serotypes					
		TIC	TCC	PIP	IPM	CFS	CAZ
Serotypes	O :1	50,0	0,0	0,0	0,0	16,6	0,0
	O :4	71,4	14,2	0,0	0,0	0,0	0,0
	O :6	50,0	0,0	0,0	0,0	0,0	0,0
	O :11	66,6	33,3	0,0	0,0	0,0	0,0
	O :15	33,3	33,3	0,0	0,0	0,0	0,0

Discussion

In this study, the tested strains of *P. aeruginosa*, have presented a high rate of resistance to the ticarcillin of 57%. Among these resistant strains, 73% were sensible to the ticarcillin+clavulanic acid. The acid clavulanic has restored the activity of this antibiotic. Contrariwise 26% of these streams were resistant in the same time, to the ticarcillin and to the tircalcillin+ clavulanic acid. These strains *P. aeruginosa* have presented also a weak resistance for the cefsulodin which is a cephalosporin of third generation. Outside of these resistance cases, all these bacterias were sensible to the piperacillin, to the imipenem and to the ceftazidim. They would not cause any therapeutic problem. This total sensibility of stumps to the imipenem has been also observed by Auajjar and al [5], people who worked on the multi-resistance to the antibiotics of *Pseudomonas aeruginosa*, *P. fluorescens* and *Staphylococus aureus* and on several hospital tissues to Bordeaux in 2006. Contrariwise, our rates don't corroborate with the rates of Kalai and Al Auajjar and al [6], who have obtained a resistance of 16% to Imipenem, of 36% to the ceftazidim in 2005 in the strains of *P.aeruginosa* isolated of the patients immunodepressed in Tunisia. These resistances of these strains might be due to the abusive using of the antibiotics in the hospital area. This abuse lead to a pressure of selection to the antibiotics. It causes the resistances often observed to the hospital. Our study beeing realized on the collected snails in the environment, there was no possible contact between the isolated strains and the antibiotics. It has been showed by the weaks resistances rate in this study. Nevertheless, the strains which have presented the resistances to the antibiotics might be considered like the strains mutated. These strains might be human that is to say coming from snail hunting industry actor (collectors, sellers, buyers).These resistant streams to the antibiotics might also have an environmental origin. The isolation of the resistant bacterias coming from the environment could be linked to a mecanism present in this area able to select the resistant clones. The variation of this resistance to the antibiotics on all sites, showed always a high rate to the ticarcillin. This resistance didn't change according the collect area. On the market, 25% of resistance observed to the cefsulodin could be linked to the repeated manipulations of the snails coming from the customers and sellers. Concerning the variation according the species of snails, the resistances heave been observed on the isolated streams of *A. achatina*, and of *A. fulica*, always for the ticarcillin molecule. It would show a particular resistance mecanism developed by these present bacterias on these two species face to this molecule. This mecanism could be enzymatic like concerning penicillin. In *A. achatina* 20% of resistance to the cefsulodin would be also linked to the market conditions, for this species is more sold on the markets. The serotypage results have showed an efficiency of 74% of serotypable streams in this study. These rates are similar to the rate got by Cattoen and al [7], (77%) in 1999 on the *Pseudomonas aeruginosa* strains isolated in 25 hospital centers in Lille. Concerning the different serotypes, our results have permitted to distinguish five groups O4(30,4%); O1(26,0%); O6(17,3%); O11(13,4%); O15(13,4%). The serogroups O2; O3; O5; O7; O8; O9; O10; O12; O13; O14; O16 have not been put in evidence in this study .These results are different from the results got by Chabaa and al., 2000 [7]. These author have isolated all the serotypes (O1 to O16) excepted the serotype O8 on the University hospital center of Rabat. This difference could be due by the fact of we have studied the environment strains. Some streams don't agglutinated have been got. These results show that it exists some strains in the snails non serotypables. Among these antigenic groups obtained, the dominant serotypes were O1 and 04.These results are different from Chabaa and all's results. These authors have got 4 majorities serotypes (O12; O2; O6 and O11). According to Hamze and al [9], the groups more frequently isolated in the patients at Lebanon in 2009 were O11(14,8%); O6(11,2%); O1(8,3%); O12(6,7%); O3(6%); O10(5,9%). Many of others works have always given the different results. Cattoen and al [7] in 1999, have showed a proportion very marked of serotypes O1; O12 in unity intensive care. According the same authors, the serotypes involved in the epidemics phenomenon's are (O11; O12). On the website of medical microbiology, actualized in 2008 [10], the serotypes O6 and O11 have been described like being the more frequently isolated in medical bacteriology. The distribution of these serotypes according the collect sites has showed a variation. This distribution is not relatively homogeny for all sites of collect mentioned by Cattoen and al in 1999 [7]. This variation showed that it would not exist any preference for example specificity of the serotypes according the collect area of the bacterias. It is also observed in different snails species. The presence of O11 only mentioned in *A. fulica* must have a particular attention, for among the serogroups involved in the hospital epidemics, only this antigenic has been proved of environment origin [4]. As this snail specie has an ubiquist character, it could be able to disseminate this serotype in the environment which could cause many epidemics. The anti-bio resistance study of these serotypes showed also the important variations. The resistance rates are different according the serotypes. These rates were also higher for all these serotypes face to the tircacillin. The resistance rate higher concerned the serotype O4. Finally, any serotype didn't present a resistance to the following molecules (piperacillin, imipenem, ceftazidim). Contrariwise, Kalai [6] and al have sowed 31 streams O11 of *Pseudomonas aeruginosa* isolated in the patients immunodepressed were all resistant to the ceftazidim in 2005.

Conclusion

In conclusion, the sensibility study to the antibiotics has been realized on 95% of the strems *P. aeruginosa* isolated. A resistance rate of 52% has been observed for the tircacillin; 13, 8% for the tircacillin clavulanic acid, 05% for the cesfulodin and 0% for the piperacillin molecules, Imipenem, ceftazidim for all streams. A rate of 74% of streams was typable, 36% have not been found serotypables. Five serotypes have been got O4(30,4%); O1(26%); O6(17,3%); O11(13,4%); O15(13,4%); O15(13,4%) precisely two O4 and O1 constitute the majority. The anti-biotic resistance study of these serotypes showed also a diversification resistance phenotypes face to the different antibiotics molecules. But, for the tircacillin molecule, all these serotypes have presented an important resistance. The rate more high concerned the serotypes O4(71,4%). The presence of these serotypes coming from the environment must have an overseeing for some of them O6 and O11 are already mentioned in the hospital pathology. The anti-biotypes and serotypes realized in this study on the streams of *P. aeruginosa* isolated of African giant snails can constitute in first approach to appreciate the sanitary quality of these snails. But these methods must be completed by the virulence genes and toxins researching.

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