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Antibiotic Resistance and Antibacterial Activity in Four Strains Isolated from Soils in Brazzaville Republic of Congo

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Abstract The antibiotic resistance and antibacterial activity of four strains isolated from soils in Brazzaville were evaluated. These four strains registered in GenBank have successively the following accession numbers: *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Bacillus subtilis* MLTBC5 (MT674681.1) and *Pseudomonas monteilii* MLTBC10 (MT674682.1). Our strains exhibited resistance according to the following profile: all four strains exhibited resistance to Fosfomicyne, two strains including *Pseudomonas aeruginosa* and *Pseudomonas monteilii* exhibited resistance to Ceftazidime, Ticarcillin + Clavulanic acid and Rifampicin while one strain in particular *Pantoea dispersa* to exhibit resistance to Amoxicillin + Clavulanic acid and to Ceftriaxone. All strains (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas monteilii* and *Pantoea dispersa*) exhibited antibacterial activity against Salmonella, three strains including *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Pantoea dispersa* exhibited antibacterial activity against -vis of *Escherichia coli*, three strains (*Bacillus subtilis*, *Pseudomonas monteilii* and *Pantoea dispersa*) exhibited antibacterial activity against *Staphylococcus aureus* and two strains (*Bacillus subtilis* and *Pantoea dispersa*) exhibited antibacterial activity against -to *Pseudomonas aeruginosa*.

Keywords: *antibiotic, antibacterial, strains, soils, resistance*

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1. Introduction

Bacteria are microorganisms that are highly adaptable to changes or disturbances that may occur in their environment. These disturbances can be, for example, a nutritional limitation or an environmental stress, but especially the presence of antimicrobial agents which could destroy them or affect their growth [1]. Indeed, since the introduction at the end of the 1930s of the first effective antibiotic and until their current excessive use, bacteria have always succeeded in setting up mechanisms or systems allowing them to bypass the action of these agents by using different resistance mechanisms just as elaborate as each other according to the new antibiotics synthesized [2]. These resistance systems cause a problem that is taking on alarming proportions due to the fact that a small number of classes of antibiotics limit the use in different treatments and that certain pathogenic bacteria are now so resistant that no treatment is possible [3]. On the other hand, it is important to note that the word antibiotic was first proposed by Sleman Waksman, a pioneer in soil screening and discoverer of streptomycin [2]. Its definition was based on the application of a chemical compound, for example, its usefulness or a laboratory effect. However, the functions or class of the compound were not taken into account. Since that time the term antibiotic has been interpreted in multiple ways, however, the best accepted definition is that an antibiotic, is a compound of biological origin which has the power to inhibit or kill microorganisms by interacting specifically with them. Their target, regardless of the class or origin of the compound [2]. Thus, when an antibiotic has the ability to induce cell death, it is described as being bactericidal. On the other hand, when an antibiotic can only inhibit cell

growth, it is considered to be bacteriostatic [4]. In order to induce either of these effects, antibiotics work on specific cell targets. The most common bacterial targets are protein synthesis, DNA, and cell wall or membranes [4].

In this work, the antibiotic resistance and antibacterial activity of four strains against four other pathogenic strains were assessed. These four strains were isolated from soils in Brazzaville, identified by molecular biology techniques and with the following accession numbers in GenBank: *Pantoea dispersa* MLTBY6 (MT646430.1); *Pseudomonas aeruginosa* MLTBM2 (MT646431.1); *Pseudomonas monteilii* MLTBC10 (MT674682.1) and *Bacillus subtilis* MLTBC5 (MT674681.1).

2. Materials and Methods

2.1. Collection Sites, Isolated Bacterial Strains and Culture Conditions

Table 1 is summurising Collection sites, used isolated and identified strains, culture conditions and geographic coordinates.

2.2. Characteristics of Pathogenic Strains

The pathogenic bacterial strains used in this work were provided by the cell and molecular biology laboratory of the Faculty of Science and Technology of the Marien Ngouabi University. Three of them have been already used by [27]. Table 2 shows these pathogenic strains and their characteristics. These strains were subcultured by the streak method on Plat Count Agar (PCA) medium and then incubated in an oven at 37°C for 24 hours.

2.3. Antibiotic Resistance

2.3.1. Choice of Antibiotics to Use

The antibiotics used in this work were chosen according to their family. Four families were chosen: the β – Lactamine family, the Aminoglycosides family, the Quinolones family, the Macrolides family and the various. Antibiotics were selected according to the strains. The antibiotic discs that have been used by family are :

- √ β – Lactamine family: Amoxicillin (AMX 25µg), Amikacin (AKN 30µg), Imipeneme (IPM 10µg), Ticarcillin + Clavulanic acid (TCC 85µg), Ceftazidime (CAZ 30µg), Ceftriaxone (PENicillin G), 10µg), Amoxicillin + Clavulanic acid (AMC 30µg), Ticarcillin (TIC 75µg).
- √ Aminoglycosides family: Tobramicin (TOB 30µg), Amikacin (AKN 30µg).
- √ Quinolone family: Ciprofloxacin (CIP 5µg).
- √ Macrolide family: Erythromycin (E 15µg), Pristinamycin (PT 15µg), Clindamycin (DA 2µg).
- √ Miscellaneous: Fosfomicyne (FSF 50µg), Rifampicin (RA 5µg), Vancomycin (VA 30µg).

2.3.2. Antibiotics Tested By Strain

The following antibiotics have been tested for *Pantoea dispersa*: AMX = Amoxicillin, AKN = Amikacin, IPM = Imipenem, CIP = Ciprofloxacin, FSF = Fosfomicyne, AMC = Amoxicillin + Clavulanic acid, CRO = Ceftriaxone. For *Bacillus subtilis*: E = Erythromycin, DA = Clindamycin, PT = Pristinamycin, CIP = Ciprofloxacin, FSF = Fosfomicyne, P = Penicillin G, VA = Vancomycin, RA = Rifampicin, TOB = Tobramicin. For *Pseudomonas aeruginosa* and *Pseudomonas monteilii*: TIC = Ticarcillin, AKN = Amikacin, IPM = Imipenem, CIP = Ciprofloxacin, FSF = Fosfomicyne, CAZ = Ceftazidime, TCC = Ticarcillin + Clavulanic acid, RA = Rifampicin, TOB = Tobramicin.

2.4. Antibiogram

An inoculum from the four strains isolated and identified from the soils was carried out. The resistance profile of the bacterial strains was evaluated by standard antibiogram using the diffusion method on Mueller Hinton medium [5].

The agar medium diffusion method was applied as standardized by the Institution of Standards for Laboratories and Clinics [6]: the test strain was streaked on PCA agar. After 24 hours of incubation at 37 °C, a pure colony of each strain was transferred to a tube containing 2 ml of sterile distilled water, the bacterial suspension of each strain was then calibrated to a turbidity approaching that of standard 0.5 from the McFarland range, which corresponds to an inoculum of approximately 1 to 2 x 10^8 CFU / ml. Ten (10) to fifteen (15) minutes later, the inoculum prepared from each strain was uniformly seeded with a sterile cotton swab by spreading over the surface of Mueller-Hinton agar (4 mm thickness of the medium per box of 140 mm in diameter previously cast). These cans were allowed to dry at room temperature for 10 minutes. The antibiotic discs were applied to the agar using sterile forceps. After incubation at 37°C aerobically for 18 to 24 hours, the diameter of each zone of inhibition was measured using a graduated ruler and compared with the reference diameters of the Antibiogram Committee of the French Society. Of Microbiology [7].

Table 1. Collection sites, strains used, culture conditions and geographic coordinates

Collection sites	strains used	Growing conditions	Geographic coordinates: Longitude and latitude
Port de Yoro	*Pantoea dispersa*	37°C for 24 hours	178.89485778543E - 85.808834987185N - 179.66937801832E - 85.775522073034N - 179.56568526223E - 85.766725306283N
Campus I	*- Bacillus subtilis* *- Pseudomonas monteilii*		
Faculty of Science and Technology	*Pseudomonas aeruginosa*		

Table 2. Characteritics of pathogenic strains used in this study

Pathogenic strains	Origin	Characteristics
Escherischia coli MN40	Urine	Résistant to penicillin and kanamycin
Salmonella typhimurium MN42	pus	Résistant to meticillin and, tetracyclin
Pseudomonas aeruginosa MN41	Waste water	Résistant to imipenem and tetracyclin
Staphylococcus auréus PM23	pus	Resitant to imipenem and tetracyclin

2.5. Antibacterial Activity

2.5.1. Preparation of the Inoculum

An inoculum of the four different identified strains was carried out in 50 ml Erlenmeyer flasks containing 20 ml of LB nutrient broth and placed in à rotary oven at the speed of 20 rpm at 37°C for 24 hours. 10ml of the solution were centrifuged at 6000trs / min for 10 minutes.

2.5.2. Antibacterial Assay

The antibacterial activity of the four strains isolated and identified from soils was tested against growth of four pathogenic strains, in particular: *staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *salmonella*. The method of diffusion in agar medium as described by [8] and taken up by [9,10]. From young colonies of 24 hours of each pathogenic strain, a bacterial suspension was made in 2 ml of sterile distilled water. The optical density of this suspension was adjusted to 0.5 McFarland modified on bench at 0.1Abs with the ZUZI brand spectrophotometer with a wavelength of 625nm. An inoculum estimated at $2x10^8$ colony-forming units per milliliter (CFU / ml) is then obtained. Using a sterile cotton swab, this inoculum was inoculated by spreading over the entire surface of the Petri dish containing Mueller-Hinton agar [7].

Each supposedly inhibitory strain was inoculated into an Erlenmeyer flask containing 20 ml of LB nutrient broth and placed in à rotary oven at the speed of 20 rpm at 37°C. for 24 hours. 10ml of the solution was centrifuged at 6000trs / minute for 20 minutes.

The sterilized blotting paper discs (6mm in diameter) were soaked in the supernatant of each strain (supposed to be inhibitory), the discs thus imbibed were delicately and under aseptic conditions deposited using sterile forceps with the surface of the petri dish. The Petri dishes are first left for 1 hour at room temperature for pre-diffusion of the substances, before being incubated in an oven at 37 ° C for 24 to 48 hours [11]. Antibacterial activity was determined in terms of the diameter of the zone of inhibition of bacterial growth produced around the discs after incubation. The zone of inhibition corresponds to the area of the agar where colonies of the pathogenic bacteria could not appear [12]. The diameters of the zone of inhibition were measured in mm using a graduated ruler.

2.5.3. Evaluation of Antibacterial Activity

The evaluation of antibacterial activity was studied by measuring the clear zone or zone of growth inhibition around the disc with a graduated ruler.

2.6. Analysis of the Results

Statistical analyses of the chart data were carried out using Microsoft Excel.

3. Results

3.1. Antibiotic Resistance

3.1.1. Demonstration of Antibiotic Resistance

Figure 1 (A, B, C, D) below presents the results of the demonstration of resistance to antibiotics of the four strains studied.

This Figure 1 shows that the inhibition diameters vary from one antibiotic family to another, and from one antibiotic to another depending on the genus or bacterial species used. From these results, we find that Fosfomicyne was resistant to all four strains used (Table 3).

In A: this image shows the results of antibiotic resistance against the strain *Bacillus subtilis*. This image shows that, *Bacillus subtilis* was resistant only to Fosfomicyne (Table 3). On the other hand, it is intermediate to all the antibiotics tested. We observe hydrolysis between ciprofloxacin and rifampicin when corking champagne.

In B: this image shows the results of antibiotic resistance against the *Pseudomonas aeruginosa* strain. This image shows that, *Pseudomonas aeruginosa* was resistant to four antibiotics (Table 3), intermediate to five antibiotics and sensitive to no antibiotics. We can see that in this image that: imipenem, ciprofloxacin and amikacin have zones of inhibition forming champagne corks.

In C: this image shows the results of antibiotic resistance against the strain *Pantoae dispersa*. This image shows that, *Pantoae dispersa* was resistant to four antibiotics (Table 3), sensitive to three antibiotics and intermediate to no antibiotic. We can see that in this image that: imipenem, ciprofloxacin and amikacin have zones of inhibition forming champagne corks.

In D: this image shows the results of antibiotic resistance against the strain *Pseudomonas monteilii*. This image shows that, *Pseudomonas monteilii* was resistant to four antibiotics (Table 3), sensitive to two antibiotics and intermediate to three antibiotics.

3.1.2. Assessment of the Antibiotic Resistance Profile

The standards of the antibiogram committee of the French society of microbiology [7] were used as references to express the results of the antibiogram of the different strains. Table 3 shows the diameters in millimeters (mm) of the zones of inhibition caused by the antibiotics with respect to the different strains, and the corresponding category (sensitive, resistant or intermediate) for each strain.

In *Pantoea dispersa*, out of a total of seven (07) antibiotics tested, including 04 from the β - lactam family, 01 from the Aminoglycosides family, 01 from the Quinolones family and 01 from the Miscellaneous. *Pantoea dispersa* was resistant to four (04) antibiotics including: Amoxicillin, Fosfomicyne, Amoxicillin + Clavulanic acid and Ceftriaxone. However, she was sensitive to Amikacin, Imipenem and Ciprofloxacin.

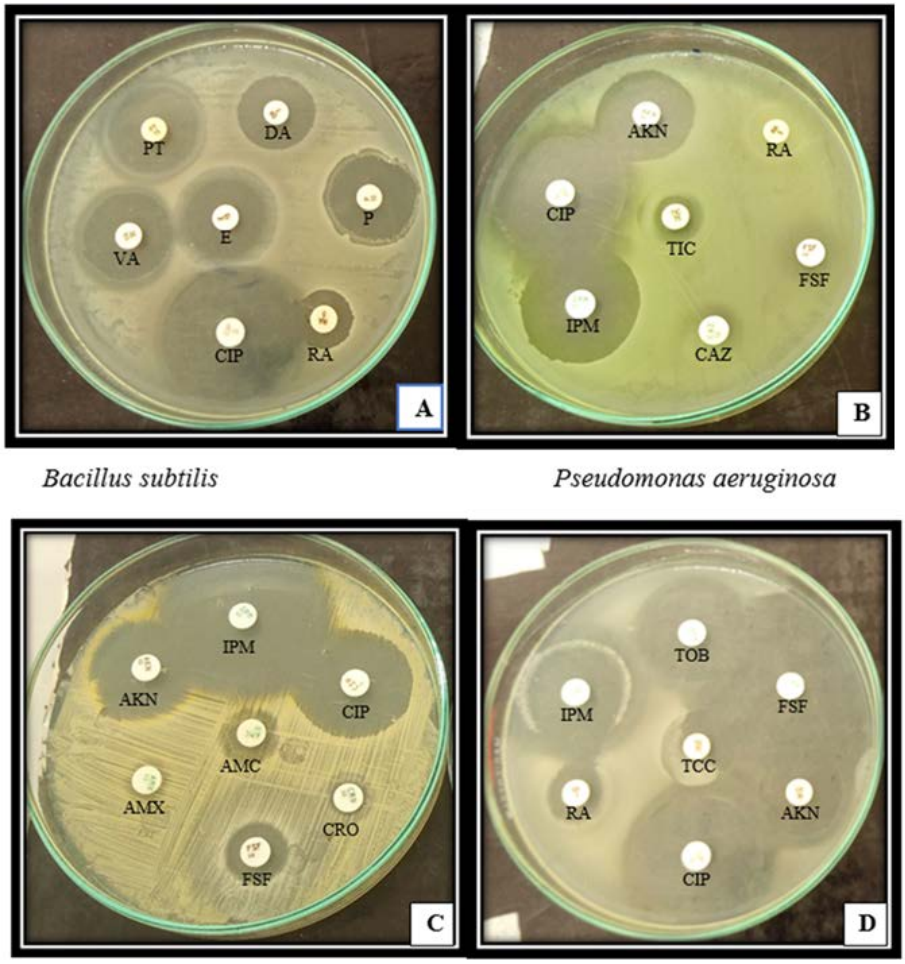

Figure 1. Antibiogram Profile of antibiotic resistance in *Bacillus subtilis, Pseudomonas aeruginosa, Pantoea dispersa* and *Pseudomonas monteilii*

Table 3. Antibiotic resistance profile in *Pantoae dispersa, Pseudomonas monteilii, Pseudomonas aeruginosa* and *Bacillus subtilis*

ATB / strains	AMX		AKN		IPM		CIP		FSF		AMC		CRO		RA		TOB	
	Diameter (mm)	Category	Diameter	Category	Diameter	Category	Diameter	Category	Diameter	Category	Diameter	Category	Diameter	Category	Diameter	Category	Diameter	Category
Pantoea dispersa	8	R	26	S	36	S	27	S	13	R	13	R	12	R	NT		NT	
ATB	TIC		AKN		IPM		CIP		FSF		CAZ		TCC		RA		TOB	
Pseudomonas monteilii	18	I	26	I	48	S	30	I	<6	R	<6	R	<6	R	<6	R	28	S
Pseudomonas aeruginosa	17	I	24	I	26	I	30	I	<6	R	11	R	<6	R	13	R	26	I
ATB	E		DA		PT		CIP		FSF		P		VA		RA		TOB	
Bacillus subtilis	24	I	20	I	24	I	30	I	<6	R	22	I	25	I	17	I	30	S

In *Pseudomonas monteilii*, out of a total of nine (09) antibiotics tested including 04 from the βeta - lactam family, 02 from the Aminoglycoside family, 01 from the Quinolone family and 02 from the Miscellaneous. *Pseudomonas monteilii* was resistant to four (04) antibiotics including: Fosfomicyne, Ceftazidime, Ticarcillin + Clavulanic acid and Rifampicin. This strain, on the other hand, was intermediate to Ticarcillin, Amikacin, Ciprofloxacin and was sensitive to Imipenem and Tobramicin.

In *Pseudomonas aeruginosa*, out of nine (09) antibiotics tested, including 04 from the β-lactam family, 02 from the Aminoglycosides family, 01 from the Quinolones family and 02 from the Miscellaneous. Pseudomonas aeruginosa was resistant to four (04) antibiotics including: Fosfomicyne, Ceftazidime, Ticarcillin + Clavulanic acid and Rifampicin. It has been intermediate to Ticarciline, Amikacin, Imipenem, Ciprofloxacin and Tobramicin. This strain was not susceptible to any antibiotics.

In *Bacillus subtilis*, out of nine (09) antibiotics tested, including 01 from the βeta - lactam family, 01 from the Aminoglycosides family, 01 from the Quinolones family, 03 from the Macrolides family and 03 from the Miscellaneous. Bacillus subtilis was only resistant to Fosfomicyne. Therefore, Bacillus subtilis was intermediate: to Erythromycin, Clindamycin, Pristinamycin, Ciprofloxacin, Penicillin G, Vancomycin, and Rifampicin, and was sensitive to Tobramycin.

From this table, we see that our strains are much more resistant to β-lactams, but were not resistant to aminoglycosides and macrolides.

Table 3 shows the evaluation of the antibiotic resistance profile in four strains (*Pantoea dispersa*, *Pseudomonas monteilii*, *Pseudomonas aeruginosa* and *Bacillus subtilis*)

(R): resistance; (I): intermediate; (S): sensitive, NT: Not tested, ATB: Antibiotics tested.

3.1.3. Antibacterial Activity

3.1.3.1. Demonstration of Antibacterial Activity

Figure 2 below shows evidence of antibacterial activity by the presence of a clear halo around the disc or well. The presence of a halo around the disc or well is synonymous with inhibition of the growth of the pathogenic strain tested.

In A: This image shows that Y6 (*Pantoea dispersa*), C5 (*Bacillus subtilis*), M2 (*Pseudomonas aeruginosa*) and C10 (*Pseudomonas monteilii*) exhibited clear halos around the discs and the well. This means that all of our strains exhibited an inhibitory effect on the pathogenic strain tested, therefore *Pantoea dispersa*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Pseudomonas monteilii* have a bacteriostatic effect on *Salmonella*.

In B: this image shows that Y6 (*Pantoea dispersa*), C5 (*Bacillus subtilis*) have halos around the discs, which means that *Pantoea dispersa* and *Bacillus subtilis* have an inhibitory effect against *Pseudomonas aeruginosa*. In contrast, M2 (*Pseudomonas aeruginosa*) and C10 (*Pseudomonas monteilii*) showed no effect against the pathogenic strain tested.

In C: this image shows that Y6 (*Pantoea dispersa*), C5 (*Bacillus subtilis*) and M2 (*Pseudomonas aeruginosa*) have halos around the discs, which means that, *Pantoea dispersa*, *Bacillus subtilis* and *Pseudomonas aeruginosa* have à vis- with respect to *Escherichia coli*. On the other hand, C10 (*Pseudomonas monteilii*) does not present a halo around the disc, therefore does not have an inhibitory effect against *Escherichia coli*.

In D: this image shows that Y6 (*Pantoea dispersa*), C5 (*Bacillus subtilis*) and C10 (*Pseudomonas monteilii*) have halos around the discs, which means that, Pantoea dispersa, *Bacillus subtilis and Pseudomonas monteilii* have à vis- with respect to *Staphylococcus aureus*. On the other hand, M2 (*Pseudomonas aeruginosa*) does not present a halo around the disc, therefore does not have an inhibitory effect against *Staphylococcus aureus*.

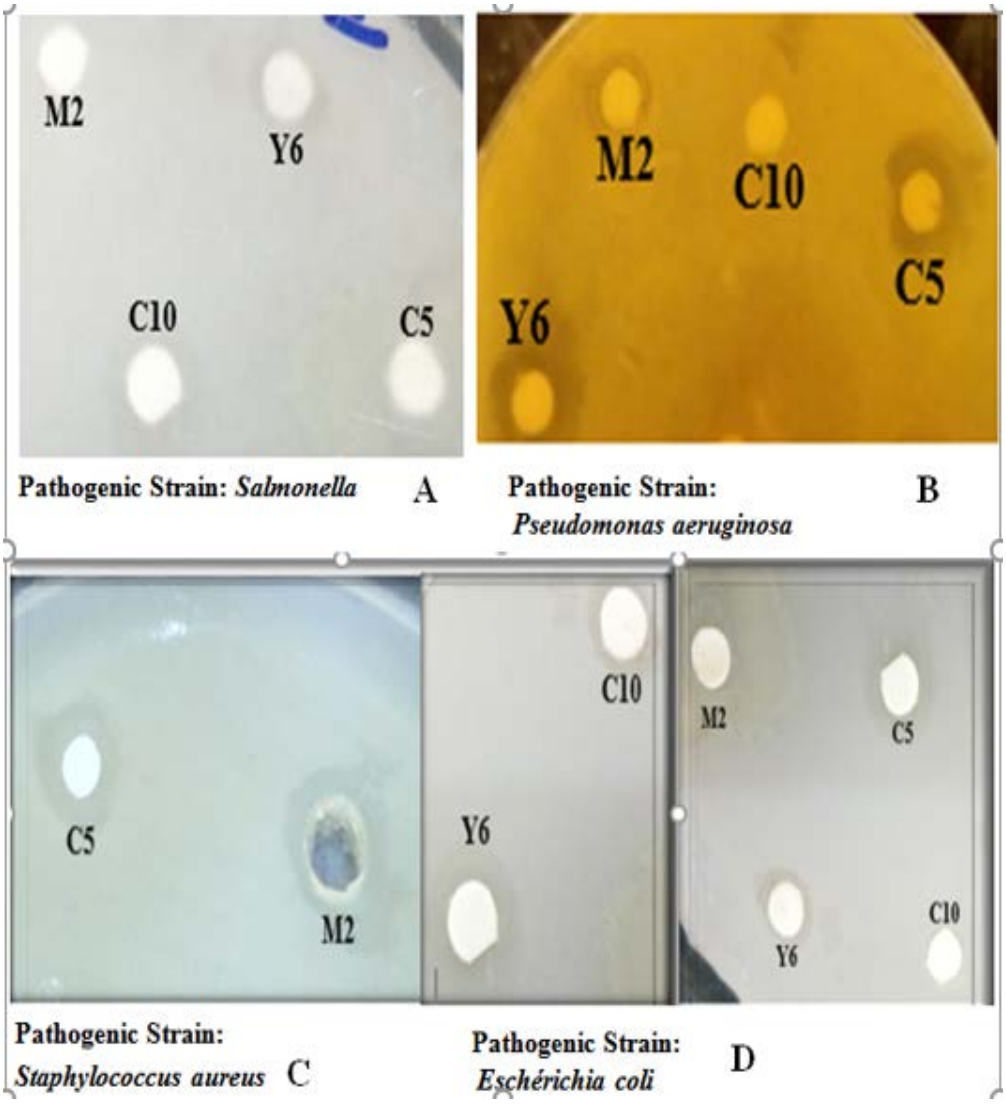

Figure 2. (A, B, C, D): Demonstration of the antibacterial activity of the four strains by a clear halo around the disc

Table 4. Antibacterial activity of bacteria isolated from soils against pathogenic strains (mm)

Strains tested / Strains of interest	OD	*Escherischia coli MN40*	*Salmonella typhimurium MN42*	*Pseudomonas aeruginosa MN41*	*Staphylococcus auréus PM23*
Bacillus subtilis MLTBC5	**0,1**	**10**	**14**	**12**	**09**
Pseudomonas aeruginosa MLTBM2	**0,1**	**08**	**14**	**00**	**00**
Pseudomonas monteilii MLTBC10	**0,1**	**00**	**12**	**00**	**09**
Pantoea dispersa MLTBY6	**0,1**	**10**	**18**	**16**	**09**

3.1.3.2. Assessment of Antibacterial Activity

Table 4 presents the evaluation of the antibacterial activity of the four strains isolated and identified from soils in Brazzaville vis-à-vis four other pathogenic strains available in the cell and molecular biology laboratory of the Faculty of Sciences and techniques of the University Marien NGOUABI in the Republic of Congo. This table shows that, at an optical density of 0.1, the diameter of inhibition varies from one strain of interest to another, from one pathogenic strain tested to another.

In view of these results, we note that: *Pantoae dispersa* and *Bacillus subtilis* exhibited inhibitory effects against all the four pathogenic strains tested. The diameters of inhibition for these two strains vary between 9 and 18mm for *Pantoea dispersa*, between 9 and 14 for *Bacillus subtilis.*

On the other hand, *Pseudomonas aeruginosa* and *Pseudomonas monteilii* only exhibited inhibitory effects against *Escherichia coli* and *Salmonella* for *Pseudomonas aeruginosa* whose inhibition diameters vary between 8 and 14mm and against *Salmonella* and *Staphylococcus aureus* for *Pseudomonas monteilii* whose inhibition diameters vary between 9 and 12mm.

4. Discussion

4.1. Antibiotic Resistance

In this study, we determined the antibiotic resistance profile and antibacterial activity of four strains isolated from soils. Indeed, it should be noted that, antibiotics act at the molecular level at one or more metabolic steps essential to the life of the bacteria. Thus, some antibiotics such as chloramphenicol and erythromycin act on target bacteria by inhibiting protein synthesis, while inhibition of peptidoglycan synthesis is the mode of action of amoxicillin and cephalosporin. It is in this perspective that we confirm that resistance to antibiotics can be explained by various mechanisms : bacteria can defend themselves against antibiotics by producing enzymes capable of inactivating them (jamming), rejecting or making themselves impermeable to antibiotics. Its penetration (efflux and shielding) or even modifying their structure (camouflage) [13,14].

Our results on antibiotic resistance in *Pantoea dispersa* show that, *Pantoea dispersa* exhibits resistance to Amoxicillin, Fosfomicyne, Amoxicillin + Clavulanic Acid and Ceftriaxone. Our results on the sensitivity of *Pantoea dispersa* to Fosfomicyne do not conform to those found by [15] who working on the Phylogeny and identification of *Pantoea* species and typing of Pantoea agglomerans strains by Multilocus gene sequencing demonstrated that Pantoae dispersa was sensitive to Fosfomicyne. This result shows that Pantoea dispersa was sensitive to ciprofloxacin, amikacin and imipenem with a broad spectrum. Of these, our results are consistent with those found by [15] who also reported that Pantoea dispersa was sensitive to Amikacin, ciprofloxacin and broad spectrum imipenem.

Our results on antibiotic resistance in *Pseudomonas* show that the two strains of *Pseudomonas*, in particular *Pseudomonas aeruginosa* and *Pseudomonas monteilii*, presented resistance to the same antibiotics tested in this study. These two strains were respectively resistant to Fosfomicyne, Ceftazidime, Ticarcillin + Clavulanic acid and Rifampicin. Our results on resistance in bacteria of the genus *Pseudomonas* to Ceftazidime, Fosfomicyne and Ticarcillin + clavulanic acid are in agreement with those found by [16] who work on resistance to colistin encoded by the mcr-1 gene in community and clinical *Pseudomonas* strains proved that, *Pseudomonas* was resistant to ceftazidime and to Ticarcillin + clavulanic acid and Fosfomicyne. Our results on resistance to bacteria of the *Pseudomonas* genus to Ceftazidime, Fosfomicyne and Ticarcillin + clavulanic acid are in agreement with those found by [17], who work on the emergence of resistance to antibiotics and correlation with the efflux pump in *Pseudomonas aeruginosa* isolated from Abidjan hospital proved that *Pseudomonas aeruginosa* was resistant to Ceftazidime and Ticarcillin + clavulanic acid. Our results on resistance to Ticarciline / Clavulanic acid in *Pseudomonas* are consistent with those found by [18] who working on the phenotypic characterization of strains of *Pseudomonas aeruginosa* isolated in the city of Yaoundé (Cameroon) have shown that *Pseudomonas aeruginosa* was resistant to Ticarcillin + clavulanin. From Table 3, we see that all the strains that were the subject of this study exhibited resistance to Fosfomicyne. According to the literature, fosfomycin, is an antibiotic that inhibits the formation of UDP-NAM from UDP-NAG, is also the target of inactivation mechanisms. Adding a thiol group to the oxirane ring of Fosfomicyne inactivates the antibiotic. There are two types of genes encoding Fosfomicyne-thiol-transferases in bacterial genomes. All are metalloenzymes whose activity depends on a cationic metal cofactor, and show strong similarities to [19]. Resistance to Fosfomicyn is due to the presence of FosA-type enzymes which are glutathione-S-transferases and which have been found in Gram-negative bacteria such as P. aeruginosa. Their activity depends on the presence of Mn^{2+} or K^{+} [20,21]. Gram-positive bacteria, which do not synthesize glutathione, have enzymes of the FosB type, using the L-cysteine residue as à thiol group donor and the Mg^{2+} cofactor. The FosB enzyme from B. subtilis has been well characterized and is very similar to the FosB enzyme identified in S. aureus [22].

Our results in *Bacillus subtilis* show that *Bacillus subtilis* was resistant only to Fosfomicyne, sensitive only to Tobramikyne, it was on the other hand intermediate to Erythromycin, to Clindamycin, to pristinamycin, to

Ciprofloxacin, to Penicillin G, Vancomycin, and Rifampicin. These results are not in accordance with those found by [24] who demonstrated that Bacillus subtilis was sensitive to Erythromycin.

4.2. Antibacterial Activity

In this study, we studied the evaluation of the antibacterial activity of four strains isolated from soils and then identified by the techniques of molecular biology of rRNA16s on the growth of four other pathogenic strains. In fact, bacteriocins act by forming pores in the cytoplasmic membrane of target cells, which leads to the release of intracellular contents and the death of the bacteria [23]. The results obtained show that our four strains generally exhibit the same spectrum of action against the pathogens tested. However, some intra-specific spectrum variations were observed on the inhibition diameters. We noted that, the strain of *Pantoea dispersa* to exhibit a larger diameter of inhibition (18mm) than others against *Salmonella* and (16mm) against *Pseudomonas aeruginosa*.

In view of our results in *Pantoea dispersa* and *Bacillus subtilis* (Table 4) we note that these two strains inhibited the growth of the four pathogenic strains tested with inhibition diameters ranging from 09 to 18mm for *Pantoea dispersa* and from 09 to 14mm for *Bacillus subtilis*. This means that, *Pantoea dispersa* and *Bacillus subtilis* have developed metabolites capable of inhibiting the growth of the pathogenic strains tested. Our results are consistent with those found by [24] who demonstrated that the *Bacillus subtilis* strains isolated from ntoba-mbodi have an inhibitory effect against the pathogenic strains tested. Our results are also in accordance with those found by [25,26] who have shown that subtle *Bacillus* strains isolated from fermented foods produce metabolites that inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*.

We also note in this study that our work on the antibacterial activity of bacteria of the genus *Pantoea* on the growth of pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* and *Escherichia coli* is the first of its kind.

Our results on the two species of Pseudomonas (aeruginosa and monteilii) show that, *Pseudomonas aeruginosa* to present an inhibitory effect against *Escherichia coli* with an inhibition diameter of 08mm and against *Salmonella* with a 14mm diameter. *Pseudomonas monteilii* on the other hand to exhibit an inhibitory effect against *Salmonella* with an inhibition diameter of 12mm and against *Staphylococcus aureus* with an inhibition diameter of 09mm. This different information sufficiently proves that the antibacterial activity is specific to each strain and cannot be extrapolated from one strain to another, even within the same species.

5. Conclusion

This study allows us to conclude that, our community strains obtained from soils, presented resistance to certain antibiotics, in particular to Fosfomicyne, to Amoxicillin + Clavulanic acid, to ceftriaxone, to ceftazidime, to ticarcillin + clavulanic acid and with Rifampicin. However, it is striking to note that the time to the emergence of resistance is very variable from one family of antibiotics to another and that within the same family this time varies considerably depending on the genera see even pathogenic species. Thus, in this study, we found that the Bacillus subtilis strain was sensitive to Rifampicin, while the Pseudomonas aeruginosa and *Pseudomonas monteilii* strains were resistant to the same antibiotic, *Pantoea dispersa* was sensitive to Amikacin, while *Pseudomonas aeruginosa* was intermediate to this same antibiotic. We also note that *Pseudomonas aeruginosa* and *Bacillus subtilis* were sensitive to Tobramicin. Finally, we note that our strains of *Bacillus subtilis* and *Pantoea dispersa* had antagonistic effects vis-à-vis the four pathogenic strains tested by inhibiting the growth of its last, *Pseudomonas aeruginosa* and *monteilii* only had inhibitory effects. On *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*.

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