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Antibiotic resistance study of some clinical strains of *Pseudomonas aeruginosa* characterization by conjugation and cleaning out of plasmid

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ABSTRACT

The antibiotic resistance of Pseudomonas aeruginosa presents a serious problem in medicine as this pathogenic germ is responsible for many diseases of which certain nosocomial infections. The 22 strains of P. aeruginosa were the target of our study as they have a multi-resistance against β-lactamines and aminosids; on the other hand, they are all sensitive to the ciprofloxacine. They all produce the β-lactamases. 15 strains are in favor of hyperproduction of céphalosporinases and all present a penicillinase phenotype. The cleaning out performed on P.aeruginosa is negative. The transfer by conjugation of the plasmid carrying gene of resistance to the ticarcilline and pipéracilline is positive for 12 of the22 studied strains. The experiments of conjugation made it possible to confirm that this resistance could have the plasmidic DNA as a support.

Keywords: *Pseudomonas aeruginosa*; β-lactamines resistance; β-lactamases; conjugation.

INTRODUCTION

Within the frame work of our study, we were particularly interested in the antibiotic resistance of *Pseudomonas aeruginosa* which is a pathogenic opportunist germ of tenimplied in the nosocomial infections. *P.aeruginosa* has an intrinsic resistance against many antibiotics; this resistance is mainly the result of a pressure selection due to abusive or bad use of antibiotics. The propagation of this resistance is elucidated in bacterial resistance by acquisition of *P.aeruginosa* of a transferable resistance to β-lactamines which presents a great risk of dissemination to other bacteria.

MATERIALS AND METHODS

The 22 strains of *Pseudomonas aeruginosa*, were isolated from various services of the C.H.U of Tlemcen hospital and a reference strain PU21 ciprofloxacine R.

After reactivation and purification by a successive subculture on BHIB then on Mac Conkey, we carried out an identification by insulation with the cétrimide agar ,by a test of growth at 42°C as well as two API20E and API20NE galleries; with an antibiogramme and a determination of the minimal concentration inhibiting (MCI) According to**[2]**; with the iodometric test detection of β-lactamases**[3]**; with the phenotypes resistance determination by the cloxacilline test ; with the plasmid transfer per conjugation ; as well as the cleaning out of plasmid by the SDS **[8]**.

RESULTS AND DISCUSSION

The identification results by API20E gallery is represented on **table1**; those of API20NE gallery on **table 2** and it proves that these strains belong to *Pseudomonas aeruginosa* species.

Figure 1: Illustration of the identification by API20E gallery

Table 1: The identification results of *P.aeruginosa* by API20E system

Tests	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMI	ARA	NO2	N2	OX
Résultats	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+

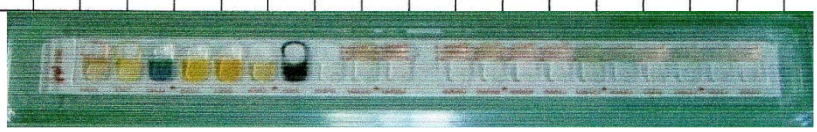

Figure 2: Illustration of the identification by API20NE gallery

Table2: Results of the identification of P.aeruginosa by API20NE system

Tests	NO3	TRP	GLU	ADH	URE	ESC	GEL	PNPG	GLU	ARA	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC	OX
Résultats	+	-	-	+	-	-	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+

Regarding the MCI results **(Table3)**, the strains tested have a multi-resistance against β-lactamines. All the strains are sensitive to ciprofloxacine which facilitated the transfer by conjugation, they are all resistant to ticarcilline and piperacilline, according to **[1]** and **[7]** this type of resistance is plasmidic, due to the enzymatic inactivation of the antibiotic. 14 strains are in favor of hyperproduction of chromosomal céphalosporinases, 15 strains have a natural resistance against céfotaxime and 16 strains are resistant to imipeneme, due to the non-penetration of the antibiotic following the loss of the porine OprD2 **[6]**.

As for the detection of the β-lactamases by the iodometric test, it reveals the production by the 22 strains of the latter which can be of chromosomal or plasmidic origin,which causes discoloration of agar around the wells **(Figure3)**.

The phenotypes of resistances determination by cloxacilline test showed a hyperproduction of céphalosporinases by 15 of our strains, which is translated by an increase in the inhibitions zones of ceftazidime and aztreonam in the presence of cloxacilline. The cleaning out of plasmid tests are negative in spite of the broad range of SDS concentrations of 400µg/ml up to 80000µg/ml whereas others, made with acridine orange and bromide of ethdium showed positive results **[4]** and **[5].** The plasmid transfer by conjugation, is positive for 12 strains, which were selected for their sensitivity to ciprofloxacine and a resistance phenotype is in favor of a penicillinase whose gene is often carried by a plasmid.

Table 3: Results of the MCI obtained for the 22 studied strains

		TIC <16>64	TCC <16>64	PIP <16>64	TAZ <16>64	CXT <4>32	CAZ <4>32	FEP <4>32	ATM <4>32	IMP <4>8	CIP <1>2	Mécanisme suspecté
A		16	16	2	2	8	1	2	4	4	0.5	Sauvage
P141	R	>512	64	128	32	16	2	4	4	2	0.25	Pase
P158	NC	>512	128	128	32	16	2	2	4	16	0.25	Pase+perte
P160	R	>512	128	128	32	16	2	4	4	2	0.25	Pase
P162	R	256	64	64	32	16	2	4	4	2	0.25	Pase
P172	R	>512	64	128	32	16	2	4	4	2	0.5	Pase
P176	R	>512	256	512	128	512	32	16	16	32	1	Pase+Case+perte
P179	R	>512	128	128	32	16	1	4	4	2	0.25	Pase
P185	R	>512	256	512	128	>512	64	32	16	32	1	Pase+Case+perte
P186	R	>512	256	>512	128	512	8	8	16	32	0.25	Pase+Case+perte
P187	R	>512	256	256	64	256	8	8	8	32	0.5	Pase+Case+perte
P201	R	>512	256	512	128	512	32	32	16	32	0.25	Pase+Case+perte
P209	R	>512	256	>512	512	>512	128	64	64	32	1	Pase+Case+perte
P230	R	128	256	256	128	512	16	32	16	32		Pase+Case+perte
P238	NC	512	256	512	128	512	16	32	16	32		Pase+Case+perte
P239	NC	>512	256	>512	64	256	16	16	16	32	1	Pase+Case+perte
P240	NC	>512	256	>512	256	>512	64	32	32	32	1	Pase+Case+perte
P241	NC	>512	256	>512	128	>512	16	16	16	32	1	Pase+Case+perte
P242	NC	>512	512	>512	128	512	16	32	16	32	1	Pase+Case+perte
P243	R	>512	512	>512	128	512	16	32	16	32		Pase+Case+perte
P250	T	256	128	64	32	16	1	4	8	4		Pase
P253	R	>512	256	>512	128	512	16	32	16	32		Pase+Case+perte
P255	R	>512	256	>512	128	512	16	32	16	32		Pase+Case+perte

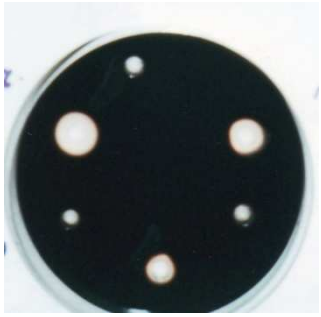

Figure 3: Iodometric test Description of the β-lactamases production

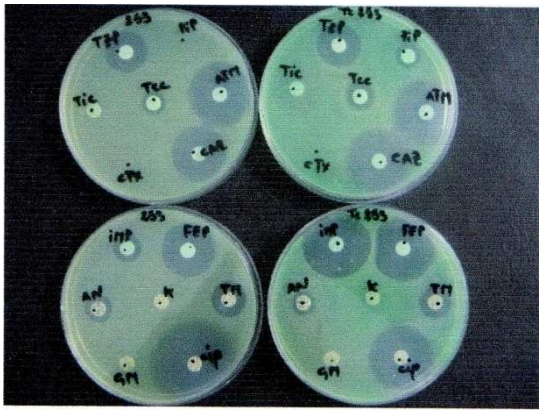

Figure 4: Illustration of the comparative antibiogramme between the donor strain and its transconjugant

The antibiogramme realized on the donors strains, the receiving strain and the transconjugants **(Table 4)** shows that these besides resistance to the ciprofloxacine, acquire other phenotypes of resistance which are inf avor of a penicillinase by the donor strain via the plasmid.

Table 4: Results of the antibiogramme

Souches	TIC	TCC	PIP	TZP	CTX	CAZ	FEP	ATH	IPM	GM	TM	AN	K	CIP
PU 21 ciproR	S	S	S	S	R	S	S	S	S	S	S	S	R	S
P 141	R	R	I	I	I	S	S	S	S	R	I	R	R	S
Tc 141	R	I	S	S	S	S	S	S	S	R	S	R	R	S
P 158	R	R	I	I	I	S	S	S	R	R	R	R	R	S
Tc 158	R	R	R	S	R	S	S	S	S	R	I	R	R	I
P 160	R	R	I	I	I	S	S	S	S	R	R	R	R	S
Tc 160	R	I	I	S	R	S	S	S	S	S	-	-	R	R
P 162	R	R	S	S	I	S	S	S	S	R	I	R	R	S
Tc 162	R	R	I	S	I	S	S	S	I	R	S	R	R	S
P172	R	R	I	S	I	S	S	S		R	I	R	R	S
Tc 172	R	I	S	S	I	S	S	S	S	R	S	R	R	S
P 179	R	R	I	I	R	S	S	S	S	R	R	R	R	S
Tc 179	R	R	I	S	R	S	S	S	S	-	-	R	R	I
P 186	R	R	R	R	R	S	S	S	R	R	R	R	R	S
Tc 186	I	S	I	S	S	I	S	S	S	R	S	R	R	S
P 187	R	R	R	R	R	I	I	I	R	R	R	R	R	S
Tc 187	R	R	I	S	R	S	S	S	S	I	-	-	R	S
P 201	R	R	I	S	R	S	S	I	R	R	R	R	R	S
Tc 201	R	I	I	S	S	S	S	S	S	R	I	R	R	S
P 209	R	R	R	R	R	R	R	R	R	R	R	R	R	S
Tc 209	R	I	S	S	S	S	S	S	S	R	S	R	R	S
P 239	R	R	R	R	R	S	S	S	R	R	R	R	R	S
Tc 239	R	R	I	S	R	S	S	S	S	R	R	R	R	I
P 253	R	R	R	R	R	S	S	S	R	R	I	R	R	S
Tc 253	R	R	I	S	-	S	S	S	S	R	I	R	R	S

Tc : transconjugants ; P : souches donatrices de *Pseudomonas aeruginosa* ; PU21ciproR : souche réceptrice.

CONCLUSION

The transfer of plasmid by conjugation, is responsible of a very important dissemination to bacterial populations which makes qualify the plasmidic resistance of contagious or of infectious. This type of transfer was described at almost all of bacterial species. It contributes for a great part to the horizontal circulation of genetic information and plays a major function in the evolution of the bacterial species.

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