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Antibacterial and antibiofilm activity of the phenazine extract of a fluorescent *Pseudomonas* on coagulase-negative staphylococci isolated from the Anti-Cancer Center of Batna, Algeria

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Abstract

Faced with many failures in the elimination of CoNS pathogens, we aimed to find an effective agent to eradicate these bacteria. In the present study we tested 20 isolates of CoNS collected from the Anti-Cancer Center of Batna (Algeria). As antistaphylococcal agents we used in our experiment 29 *Pseudomonas* isolates selected from the roots of plant (*Hordeum murinum*). The results of crossed streak method on MHA medium showed that the 29 isolates of *Pseudomonas* inhibited 19 CoNS. The strain with the greater inhibitory effect PK inhibited 14 CoNS. The high performing *Pseudomonas* PK has been used for the production of phenazine compounds. The dry compound of PK from ethyl acetate extraction has been shown to be effective against 13 isolates of staphylococci. The percentage of biofilm inhibition by the PK phenazine extract was between (32.2%) and (77.56%). The analysis of the compound obtained by UV-visible and infrared spectrum showed that it was similar to hydroxyphenazine.

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Introduction

Coagulase-negative staphylococci (CoNS) are among the most frequently isolated microorganisms in microbiological samples. These bacteria, long regarded as contaminants, are currently recognized as true pathogens (Bertrand *et al*., 2002; Koksal *et al*., 2009) including species *S. epidermidis*, *S. haemolyticus* and *S. saprophyticus*, CoNS are causative agents of conjunctivitis, endophthalmitis, skin infections, urinary tract infections, endocarditis, peritonitis, bone and joint infections, post-neurosurgical meningitis, infections originated from equipments and valves as well as septicemia whose starting point can be a catheter (Garnier and Denis, 2007).

Many antibiotics are available for the treatment of various pathogenic bacteria. However, the increase in antibiotic resistance has led to more severity of diseases caused by CoNS. In addition, low immunity in hosts and the ability of bacteria to develop antibiotic resistance associated with biofilm have further increased the number of life-threatening bacterial infections in humans (Raut and Karuppayil, 2014). Consequently, treatment choices for resistant CoNS infections extend to natural antibacterial (Singh *et al*., 2015; Moloney, 2016). The capacity of the antibacterial compounds obtained from other microorganisms to inhibit CoNS has also been tested (El Amraoui *et al*., 2014). Among the metabolites studied produced by various groups of microorganisms those of *Pseudomonas* (Chain and Mellows, 1977). The members of this bacterium are found in the different environments, including soil, water, plant surfaces, animals and plants. They are also competitive colonizers in the rhizosphere and are well adapted to different biotic and a biotic stresses. *Pseudomonas* is well known for its ability to use a variety of organic compounds and for its production of antimicrobial compounds some of them are used in the treatment of bacterial diseases (Laine *et al*., 1996). It also shows activity against staphylococci (Cardozo *et al*., 2013; Hotterbeekx *et al*., 2017). *Pseudomonas fluorescens* is a non-pathogenic saprophyte that colonizes water, soil and plants surfaces. It lives in a commensally relationship with plants, allowing them to acquire essential nutrients, degradation of chemicals and biological pollutants species (Igbinosa *et al*., 2014). *P. fluorescens* also produce a large number of secondary metabolites that could play a role in the antagonistic effect in soil (Jacques *et al*., 1993) making the bacteria a performing biocontrol agent (Igbinosa *et al*., 2014). Among its metabolites produced are phenazines which have long been recognized for their importance in microbial destruction and their positive physiological roles for the bacteria that produce them are increasingly appreciated (Grahl *et al*., 2013).The aim of this study is to isolate, from plant rhizospheres, a set of fluorescent *Pseudomonas* to examine their antagonist and antibiofilm activity against clinical isolates of CoNS. This step will lead us to search for an extracellular metabolite effective against staphylococci of medical interest.

Materials and methods

Bacterial material

20 CoNS isolates were collected between January 1 and February 28, 2017 from hospitalized patients in different departments (Onco-Pediatric, Onco-Hematology, intensive care, Carcinological Surgery) of the Anti-Cancer Center of Batna (Algeria) and non-hospitalized patients who came for external consultation.

Clinical strains of CoNS were grown on Chapman medium and identified using standard bacteriological methods. CoNS were used as target microorganisms to determine the antagonistic activities and spectrum of action of *Pseudomonas* isolates.

Antibiotic susceptibility and minimum inhibitory concentrations (MIC) of CoNS strains

Antibiotic sensitivity of CoNS isolates was achieved by the MHA disk diffusion method, as described by Bauer *et al*. (1966). The diameters of the inhibition zones were measured with caliper. The results were interpreted according to the recommendations of Clinical and Laboratory Standards Institute (2014).

MICs were determined for Oxacillin and Vancomycin on MHA using E-test method and interpreted

according to Clinical and Laboratory Standards Institute (2014).

Study of biofilm formation by the in vitro crystal violet staining method of on microplates (TCP)

Polystyrene microplates with 96 wells were used to study adherence and biofilm formation in CoNS isolates. The quantitative determination of biofilm formation in the microplates was evaluated according to Christensen *et al.* (1985) method with some modifications. Briefly, the CoNS were grown in nutrient agar medium for 18-24h at 37°C; one colony of each isolate was suspended in 5ml of TSB and incubated at 37°C for 24 h. Each suspension is diluted 1 / 100th in the same TSB + 1% glucose and each well of the microplate was filled with 200µL of this dilution (three independent cultures for each species were used). A sterile TSB + 1% glucose was used as a control. These microplates were covered and incubated for 24 h at 37°C. The contents of the wells were gently poured and washed three times with sterile physiological water, dried in an inverted position in an oven at 60°C for 60 min, adherent cells are stained with 200µl of 1% crystal violet (w/v). After 30 min of incubation, excess of crystal violet was removed by 5 successive washes with sterile distilled water. The dye incorporated by the adhered or biofilm-forming cells was solubilised with 200µL of 95% ethanol (v/v).The amount of solubilised crystal violet was measured by reading the OD at 550nm (Rodrigues *et al.,* 2010). The interpretation of the results was performed according to Stepanovic *et al.*, (2007) recommendations.

Isolation of Pseudomonas

Several samples were taken from different points in the vineyards of two regions of the state of Mostaganem, one of Oued Elkhire and the other of Sidi Lakhdar on February 2018. Samples are wild grass roots (barley of rats = *Hordeum murinum*). The isolated strains were identified using conventional bacteriological methods. Only the colonies giving a fluorescent yellow-green pigment on King B medium were selected (Bossis *et al.*, 2000).

In Vitro Antagonism Test

A cross streak method as described by Selvin *et al.*, (2009) was used. It consists in seeding the *Pseudomonas* antagonist strain in a single streak at the edge of the surface of MHA plate. After incubation for 48h at 30°C, *Staphylococcus* isolates are seeded perpendicular to *Pseudomonas*. After 24h further incubation, the antimicrobial interactions are analyzed by measuring the inhibition zone size using a caliper.

Inhibitory effect of Pseudomonas culture supernatants

The method used in the present work described by Veerendra kumar and Janakiram (2015) is based on the diffusion of the inhibitory agent into wells made in an agar containing an indicator strain. The culture supernatant of *Pseudomonas* was obtained after centrifugation of a culture in MHB, the supernatant was filtered through a millipore filter of 0.22µm. 0.1ml of a pure culture of 24 h indicator strain, whose optical density located between 0.08 and 0.1 at λ = 625nm was placed in each Petri dish containing 20ml of MHA. Wells of 4 mm of diameter were made, and then each well was filled with 50µL of supernatant and incubated at 37°C for 24 h. The inhibition zones were revealed around the wells.

Production and extraction of phenazines

The phenazine compounds were extracted according to the method described by by Bonsall *et al.*, (1997) and Dahah *et al.*, (2016). The performing isolate PK was inoculated on solid King B medium and incubated at 30°C for 24h. Flasks containing 50ml of Nutrient Broth Yeast Extract (NBY) were inoculated with the PK isolate, and then incubated at 30°C for 72 h with continuous shaking of 180rpm. Then cultures were centrifuged and the supernatant was acidified to pH 2 with concentrated HCl (Delaney *et al.*, 2001). Phenazines were extracted from the supernatant by ethyl acetate 1(v/v). The organic phase was treated with anhydrous ammonium sulfate, filtered and then concentrated to dryness under reduced pressure using a rotary evaporator at 55°C (Mezaache-Aichour *et al.*, 2012).The dry substrate is solubilised separately in methanol and in DMSO 10% for subsequent utilizations (Delaney *et al.*, 2001).

Study of the antimicrobial activity of the extract by the disk method

Antibacterial activity of the extract obtained, was determined using a diffusion disc method of Hazalin *et al.*, (2009). Two solutions of 0.25g/ml and 0.5g/ml of the dry substrate were prepared in DMSO 10%. 0.1ml of inoculum of each of the 20 isolates of CoNS was swabbed on MHA plate. The sterile disks (6mm in diameter) were deposited and impregnated with 10µl of the extract. The Vancomycin disk (30µg) was used as a positive control and the DMSO 10% disk as a negative control. Then, the dishes were incubated at 37°C for 24 h.

MICs of phenazine extract

The determination of the MICs of The phenazine extract against the CoNS was carried out according to the dilution technique in liquid medium described by (Bazargani and Rohloff, 2016) with some modifications. The bacterial strains were cultured on MHA and incubated at 37°C for 12 h, after incubation, 5-7 isolated colonies were inoculated in tubes containing 5ml MHB and incubated at 37°C for 8 to 12 h, then, the bacterial suspension was diluted 1: 100 in sterile MHB (10^6 CFU/ml). For each microplate well, 100µL of MHB were added, then 100µL of the extract was placed in the first microplate well and two-fold serially diluted was performed in MHB. Finally, 100µL of the diluted bacterial strain was added to obtain a final concentration between 250 and 1.95mg /ml. 100µL of DMSO 10% was used as a negative control with 100µL of MHB. *S. aureus* ATCC 25923 was used as a control.

After incubation for 18 to 24h at 37°C, the MIC was determined macroscopically via turbidity observation (Nostro *et al.*, 2016). Minimum bactericidal concentration (MBC) was determined by inoculating 10µL of all MICs on MHA (Marino *et al.*, 2010).

Effect of phenazine extract on biofilm formation

The extract at the MICs concentration was evaluated for their inhibitory potential against cell attachments described by Bazargani and Rohloff (2016). 100 µl of the extract at the MICs value were added to each well of a 96-well microplate. The negative control contained 100 µl of TSB + 1% Glu. Finally, 100 µl of each bacterial culture (10^6 CFU/ml) were put into each well. 200µl of TSB + 1% Glu + DMSO 10 % were added without bacterial culture and a blank control (TSB + 1% glucose + extract) were included. The microplates were incubated at 37°C for 24h. Then, the biomass biofilm was assayed using the crystal violet staining test as described above. The mean absorbance of the samples was determined, the absorbance in blank well was subtracted from absorbance reading and percentage inhibition and efficiency were determined.

UV-visible spectrophotometer analysis

Phenazine analysis by UV-visible spectrophotometer described by Veselova *et al.*, (2008) was assayed. The dry phenazine extract was put into a methanol solution and then put into a vat for spectrophotometer analysis in order to characterize the absorption spectrum of this metabolite.

Fourier Transform Infrared Identification (FTIR)

A sample of the extract was analyzed by a Fourier Transform Infrared spectroscopy technique in order to characterize partially the structure of the extracted phenazine (Dahah *et al.*, 2016).

Results and discussion

Identification of CoNS

We identified 20 isolates of CoNS belonging to 5 different species following conventional methods. (45%) of CoNS studied were belonged to, *S. epidermidis,* (30%) to *S. haemolyticus,* (15%) to *S. xylosus*, (5%) to *S. hominis* and finally, (5%) too to *S. cohnii*. Similar study on blood culture samples reported by Koksal *et al.* (2009). Ehlersson *et al.* (2017) found different results. A dozen of species and subspecies, most of which part of the commensal flora, are potentially pathogenic for man through the breaking of the cutaneous -mucous barrier. *S. epidermidis* may be responsible for vascular or joint prosthetic infections, heart valves, CSF bypass valves; it is also isolated from peritoneal dialysis, peritonitis, subacute endocarditis treatment and from intravenous drug users (Abalain-Colloc *et al.*, 2014). *S. haemolyticus* is the second most commonly isolated of CoNS. It is more frequently associated with endocarditis on native valves, bacteraemia, peritonitis and central venous catheter infections (Herard *et al.*, 1998). It can be responsible for urinary tract infections (Gaucherie and

Avril 2005; Nauciel and Vildé 2005). *S. xylosus* is rarely isolated in blood cultures, rare reports of human infections have been reported, including endocarditis, pyelonephritis, and intra-abdominal infection (Mack *et al.*, 2006).

Antibiotic resistance of CoNS

The isolates are resistant to antibiotics with different percentages (Table 1). Different results were found by Pinna *et al.* (1999) of which (67%) CoNS are Penicillin-resistant. No strain of CoNS showed resistance to Vancomycin. Similar results are also reported by Jain *et al.* (2004). Different findings are reported by Soumya *et al.* (2017) with a (7%) resistance to Vancomycin. According to Leclercq (2002), the strains with diminished susceptibility to glycopeptides appear to be rare but may be underestimated because of the difficulty of *in vitro* detection of resistance. Results showing deference with our findings have been reported by Shrestha *et al.* (2017) for Amikacin with a sensitivity of (67%) and for Chloramphenicol (12.6%) and (8.9%) were reported by Cuevas *et al.* (2004) in Spain by statistical studies of the years 1996 and 2002 successively.

Table 1. Antibiotic resistance and MIC of CoNS.

Antibiotics	Percentage (%)
Penicillin	100
Cefoxitin	90
Fucidic acid	65
Erythromycin	55
Clindamycin	25
Pristinamycin	40
Amikacin	10
Kanamycin	50
Gentamycin	35
Chloramphenicol	00
Rifampicin	40
Teicoplanin	00
Ofloxacin	20
Levofloxacin	35
Ciprofloxacin	35
Trimethoprime/Sulfamethoxazole	35
Tetracyclin	05
Vancomycin	00
Oxacillin	75

Detection of CoNS biofilm formation by in vitro microplate crystal violet staining (TCP) method

In the TCP method, biofilm production was detected in all isolated CoNS (100%) with different intensities: (35%) are highly biofilm producing, (25%) are moderate and (40%) are low. Soumya *et al.* (2017) found that (11%) of CoNS strains were strongly biofilm forming and (6%) were moderate. Other different results have been reported by Nasr *et al.* (2012).

Isolation of Pseudomonas isolates and identification of PK isolate

In our study we selected 29 isolates cultivated on King B medium. Their preliminary identification was essentially based on morphological characteristics, Gram stain, mobility, catalase search and oxidase. These characters allowed us to classify them with the group of fluorescent *Pseudomonas* (Bossis *et al.*, 2000). Results of the *in vitro* antagonism tests Showed that inhibition was variable depending on the *Pseudomonas* isolate and the strain of CoNS tested (Fig. 1).

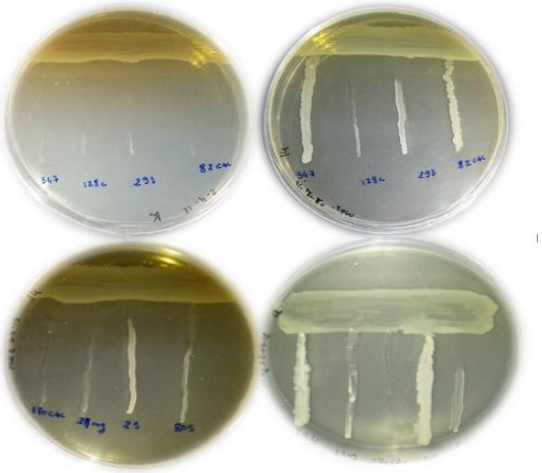

Fig. 1. *Pseudomonas* antagonistic activity against CoNS isolates.

Overall, the results of this test showed that all 29 *Pseudomonas* showed a growth inhibition of 19 of the targeted Staphylococci (Table 2). Clear areas between *Pseudomonas* and the most of Staphylococci tested were observed; these areas vary from 3 to 55mm, the histograms given by (Fig. 2) show the different inhibition zone distances in mm between *Pseudomonas* isolates and Staphylococci. The largest area was noted by the PK isolate against *S. epidermidis* 94C. The second histogram given by (Fig. 3) shows the number of Staphylococci inhibited by each Pseudomonas isolate. 14 different Staphylococci were inhibited by the PK isolate,

while the PR isolate inhibited only 6 *Staphylococcus* could have an effect on *S. epidermidis* 203CAC which is Resistant to all other isolates of *Pseudomonas,* reflecting the difference of the inhibitory substances responsible for antagonistic activity in each strain. The identification of PK isolate by API NE Gallery directs us towards the species *P. fluorescens*.

Table 2. Antagonism test results by crossing technique.

	S. epidermidis										*S. haemolyticus*				*S. xylosus*				*S. cohnii*	*S. Hominis*	
	29 mg	94 C	170 CAC	89 3	203 G	173 C	82 C	128	123 C	805	182 H	46 7	1076	316 H	14:15	25	293	347 H	106 CAC	132	T
PK	+	+	-	+	-	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	14
P25	+	-	+	-	-	+	+	+	-	+	-	+	-	-	+	-	-	-	+	+	10
P14	+		-	-	-	+	-	+	-	+	+	-	-	-	-	+	+	-	+	-	8
PI	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	-	-	7
P19	+	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	+	6
P10	+	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-	6
PA	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	6
PR	-	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	6
P26	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	+	5
PL	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	5
PB	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	4
PE	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	4
PN	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	4
PQ	-	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	4
PG	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	4
P15	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	4
P9	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	3
P11	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	3
P3	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	3
PJ	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	3
PS	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	3
P22	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	2
P1	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	2
PH	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
P28	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
P21	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
P12	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
P17	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
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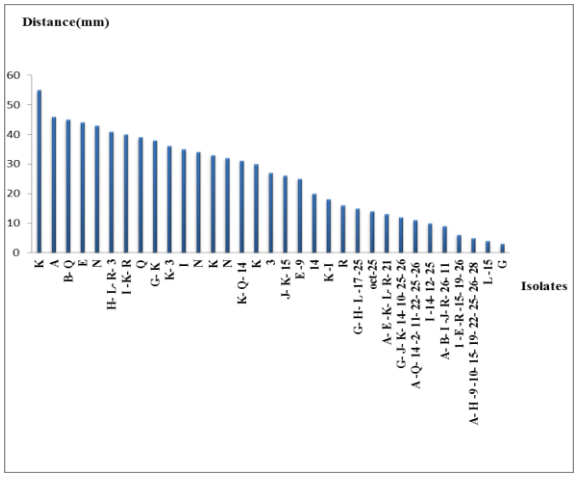


Fig. 2. Distances of inhibition zones between *Pseudomonas* isolates and Staphylococci.

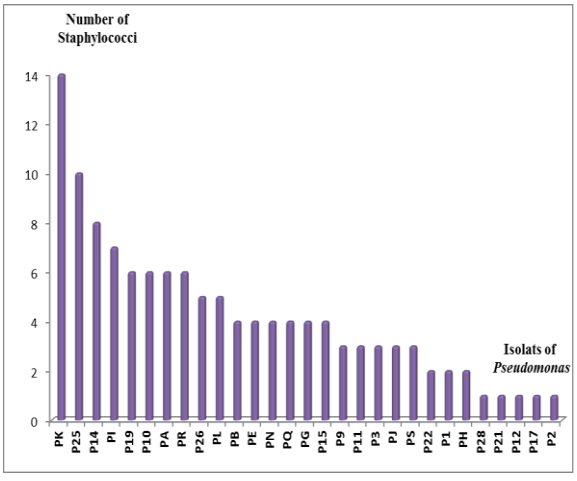


Fig. 3. Number of Staphylococci Inhibited by each *Pseudomonas* Isolate.

Some strains of fluorescent *Pseudomonas* that colonize agricultural soils have several intrinsic characteristics that make them particularly attractive for use as biological control agents (Haas and Keel, 2003). In addition to competition for carbon sources, antagonism can be attributed largely to the production of secondary metabolites (antibiotics, siderophores, hydrogen cyanide, enzymes etc.) (Jacques *et al.*, 1993). Indeed, *P. aeruginosa* produces more than 55 quinolones/quinolines in addition to 2-heptyl-3-hydroxy-4 (1H) -quinolone, and these have significant antibiotic activity against gram-positive bacteria. Antimicrobial quinolones can be inserted in extracellular membrane vesicles to cause a *S. epidermidis* lysis (Mashburn *et al.*, 2005). Another extracellular protein secreted by *P. aeruginosa* which has notable staphylolytic activity is the LasA protease. Extracellular polysaccharides secreted by *P. aeruginos*a may be a promising strategy for use against staphylococcal biofilms in future applications (Qin *et al.*, 2007).

The inhibitory effect of the culture supernatant of Pseudomonas

Among the 29 isolates with antagonistic activity, 12 isolates namely: PK, P14, PG, PL, PN, PR, P3, PE, P25, PJ, PQ and PA were selected because of their inhibition spectrum against *Staphylococcus* and the zones of their inhibition. The 12 isolates selected were tested for the inhibitory effect of their supernatants. However, they did not show any antagonist activity. This may be justified by the low concentration of inhibitory agents in the supernatants. According to studies by Emmerich and Löwthe cell-free culture fluid of *P. aeruginos*a should be concentrated to one tenth of its initial volume to be effective (Leisinger and Margraff, 1979).

The antimicrobial effect, MICs and BICs of phenazines extract

Phenazine production by PK isolate was performed on NBY medium, and extraction was by ethyl acetate. The dry phenazine extract was then melted in a (DMSO 10%) solution to test its antimicrobial effect. The results obtained showed that our extract exhibits anti staphylococcal activity, as shown in (Fig. 4) clear areas around the disks impregnated with the phenazine extract were observed. These zones of inhibition vary according to the strain tested between 6.75 ±0.353 and 27.33±0.707 mm (Table 3). The largest zone was found against isolate 347 H (*S. xylosus*).

Pseudomonas produce a range of phenazine compounds that differ widely in their antibiotic properties, depending on the nature and position of the side groups attached to the phenazine nucleus (Saleem *et al.*, 2010). Other biological activities of phenazine include natural products such as anti tumorals, anti malarials and antiparasitics have been reported (Laursen and Nielsen, 2004). Our results are in agreement with the studies of several authors, who showed that phenazine substances are known for their antifungal and antibacterial activities (Shahid *et al.*, 2017).

In the present study, the MICs were tested to know the ability of phenazines to inhibit the growth of 13 CoNS strains. This MIC of phenazine extract was from 3.9mg/ml to 31.25mg/ml. The MBC was ranging from 7.81mg/ml to 31.25mg/ml (Table 3).

According the studies of Nansathit *et al.* (2009), phenazine-1-carboxylic acid showed more potent inhibition against some strains such us: *A. avenae* subsp *citrulli*, *B. subtilis*, *C. albicans*, *E. coli* and *X. campestris* pv. *vesicatoria*. The inhibitory mechanism of phenazines was the result of the toxicity of the superoxide radical and hydrogen peroxide, described in a report by Dwivedi and Johri (Nansathit *et al.*, 2009). According to other studies conducted by Borrero *et al.*, (2014), 13 various phenazine compounds, five of which are of natural origin, possess inhibitory activity against *S. aureus* and *S. epidermidis*. Phenazine-1-carboxylic acid and chlororaphine showed only weak antibiotic activity against *S. aureus* (MIC> 5µg/ml) (Laursen and Nielsen, 2004).

Effect of phenazine extract on CoNS biofilm formation

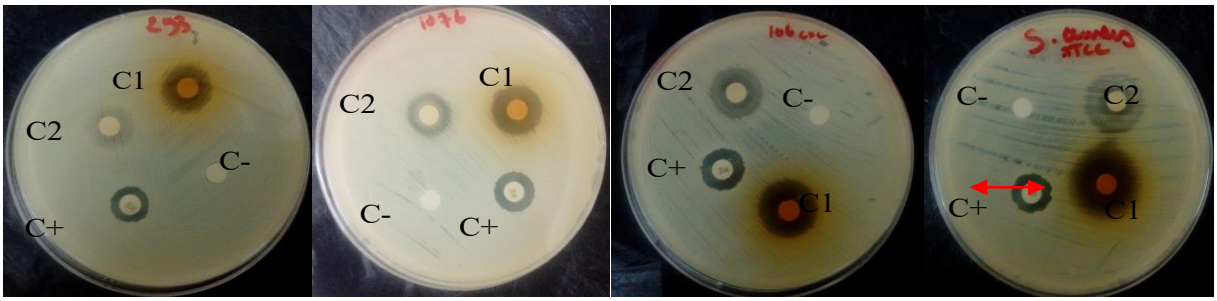
In our study, the phenazine extract showed antibiofilm activity with significant reduction between (32.2%) and (77.56%) (Table 3). Staphylococci commonly colonize the skin and are

frequently found in wounds and implants. Interestingly, *S. epidermidis* was not considered an opportunistic pathogen until the widespread use of medical devices. Biofilm formation, then, can be thought of a virulence factor a bacterial strategy that contributes to its ability to cause an infection (Hall-Stoodley *et al.*, 2004). Qin *et al.* (2009) found that *P. aeruginosa* extracellular products, mainly polysaccharides, disrupted established *S. epidermidis* biofilms and these extracellular products are important microbial competition factors that overcome competition with *S. epidermidis*, and the results may provide clues for the development of a novel strategy for controlling *S. epidermidis* biofilms.

Table 3. Diameters of the zones of inhibition (mm), MICs, BICs and biofilm inhibition of phenazine extract of PK strain against isolates of CoNS.

Isolate number	Bacteria tested	Diameter of zones Inhibition (mm)		MIC	BIC	BIC /MIC	Biofilm inhibition
		Concentration of 0.5 g /ml	Concentration of 0.25 g /ml	(mg/ml)	(mg/ml)		(%)
106CAC	*S. cohnii*	18.5±0.5	11±0	7.81	7.81	1	32.20
316H	*S. haemolyticus*	14.83±0.288	-	15.62	15.62	1	53.39
347 H	*S. xylosus*	27.33± 0.707	17.33±0.288	3.9	7.81	2	70
82CAC	*S. epidermidis*	13.5±0.5	8.5±0	7.81	15.62	2	77.56
293	*S. xylosus*	13.5±0.5	-	15.62	15.62	1	71.31
805	*S. haemolyticus*	10.67±1.040	-	15.62	15.62	1	30
25	*S. xylosus*	12±0.5	-	31.25	31.25	1	70.67
123	*S.epidermidis*	16.67±0.577	08±00	15.62	15.62	1	66.79
1076	*S. haemolyticus*	17±01	12±00	7.81	15.62	2	75
128	*S. epidermidis*	14.5±0.5	7.83 ± 0.288	15.62	31.25	2	76.56
29mg	*S. epidermidis*	19.67±1.443	-	31.25	31.25	1	61.11
170CAC	*S. epidermidis*	13.67±0.577	6.75±0.353	7.81	15.62	2	69.38
467	*S. haemolyticus*	16.67±0.288	-	15.62	15.62	1	61.90
S. aureus	*S. aureus* ATCC 25923	16.16±0.288	08±00	7.81	15.62	2	Non tested

-: non inhibited



C1: concentration 0.5 g /ml, C2: concentration 0.25g /ml, C (-): negative control (DMSO 10%), C (+): positive control (Van 30µg).

Fig. 4. Antimicrobial effect of the phenazine extract on the target bacteria.

Characterization and identification of the extracted metabolite

The dry phenazine extract was disolved in a methanol solution, and after spectrophotometric analysis by UV-visible spectrophotometer we

obtained a single peak. The peak obtained is shown in (Fig. 5). The extract showed absorbtion characteristics at about 220nm.

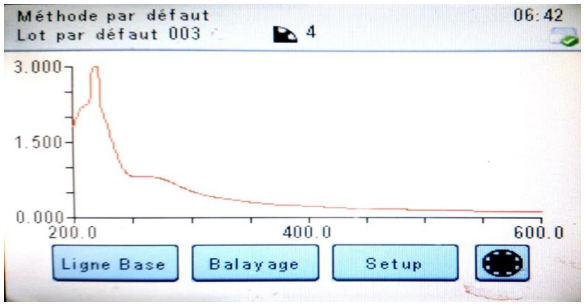


Fig. 5. Spectrum of phenazine identification by UV-visible spectrophotometer.

Fourier Transform Infrared Identification (FTIR)

The IR spectrum obtained represents the characteristic vibrations of the various bonds constituting the structure of the extract studied (Fig. 6). The majority of peaks appear in the 400 to 4000cm-1 range. The absorption bands correspond to the molecule whose details are as follows:

A band at 3664cm-1 corresponds to the elongation of the O-H group for phenols;

A band located at 2924cm-1 corresponds to the elongation of the aromatic C-H group;

A band located between 1664cm-1 corresponds to the elongation of the aromatic C = N group;

A band at 1401cm-1 corresponds to the elongation of the C-N group;

A band at 1228cm-1 corresponds to the C-C elongation;

A band at 1072cm-1 corresponds to the elongation = C-O.-

The different bands detected correspond to the molecule of hydroxyphenazine.

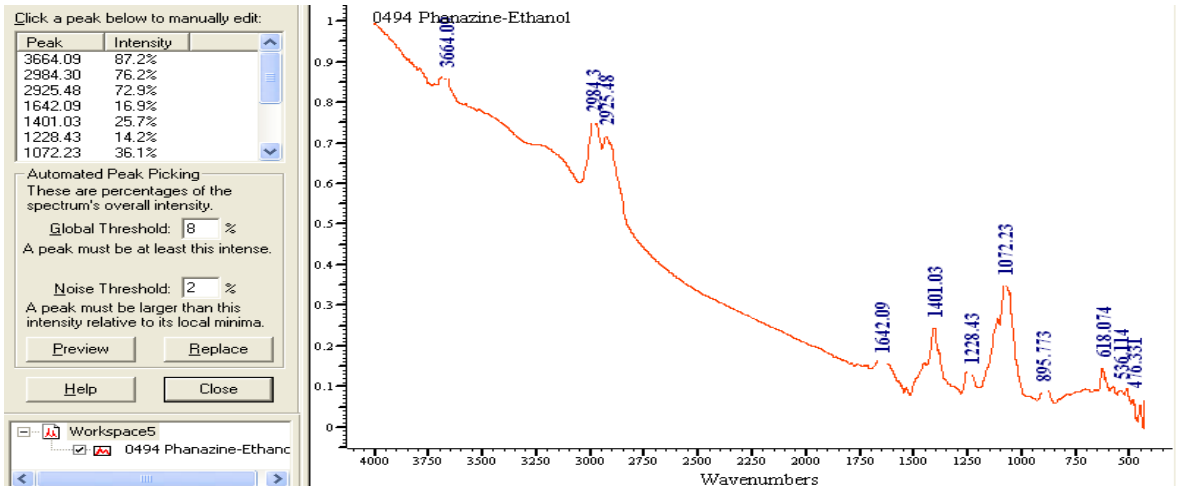


Fig. 6. Spectrum of phenazine identification by FTIR.

Conclusion

The aim of the present work is to isolate a group of fluorescent *Pseudomonas* from rhizosphere soil (which is known for its high ability to synthesize secondary metabolites) to control CoNS. Isolates from the Anti-Cancer Center belong to the following species: *S. epidermidis*, *S. haemolyticus*, *S. xylosus*, *S. hominis* and *S. cohnii*. These isolates are all susceptible to these antibiotics: Chloramphenicol and Vancomycin. The 29 *Pseudomonas* from the root samples of the wild *Hordeum murinum* were antagonized on MHA, in order to evaluate their antistaphylococcal capacity against 20 clinical isolates of CoNS. Thus, *Pseudomonas* isolates tested, revealed a power of inhibition on 19 CoNS. These zones of inhibition vary from 3 to 55mm, the largest zone has been observed in the PK isolate against *S. epidermidis* isolate No. 94C. 13 different CoNS were inhibited by the PK isolate. The other *Pseudomonas* inhibited a significant number of *Staphylococcus* with diversity in their spectrum. Therefore all CoNS (except one isolate) were inhibited by all *Pseudomonas* isolates combined. This reflects the difference of the inhibitory substances responsible for the antagonist activity in each strain. The obtained dry compound of PK has been shown to be effective against staphylococci and. The MICs and MBCs values

of the phenazine extract showed a remarkable activity. The percentages of its inhibition of the CoNS biofilms were relatively elevated. Analysis of the compound by UV-visible spectrum and infrared has shown that it is probably close to hydroxyphenazine.

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