Antibiotic Resistance Pattern of *Acinetobacter baumannii* Strains Isolated from Different Clinical Specimens and Their Sensibility Against Bioactive Molecules Produced by *Actinobacteria*

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Abstract

Acinetobacter baumannii frequently targets the most vulnerable hospitalized patients, especially in intensive care units. Bacteremia, nosocomial pneumonia and surgical wound infections caused by *A. baumannii* have a higher mortality compared to other nosocomial pathogens. The severity of the diseases caused by this microorganism is accentuated by its resistance to many antibiotics currently used in the world. In this context, we undertook to isolate several *A. baumannii* strains from various samples collected in the Béni Messous University Hospital Center, Algiers (Algeria), to determine the antibiotic resistance profile of these strains and to evaluate the antagonistic potential of some actinobacterial strains against *A. baumannii*. Nineteen *A. baumannii* strains were isolated and identified. The predominant number of strains isolated from pulmonary samples (36.84%) and from the intensive care unit (57.89%) was *A. baumannii*. All *A. baumannii* strains were sensitive to colistin but exhibited high resistance to 11 other antibiotics tested. Of the 19 strains, 15 were metallo- β -carboxypeptidase producers, 10 were cephalosporinase, and three were extended-spectrum β -lactamases (ESBL) producers. Of 59 strains of actinobacteria tested, 19 (32.20%) showed activity against at least one strain of *A. baumannii*, the highest activity being detected in strains belonging to the genus *Saccharothrix* (or related genera). However, *Streptomyces* sp. WAB9 had the widest spectrum of activity. Based on the obtained results, colistin remains the unique antibiotic which inhibits the growth of all *A. baumannii* strains, and some actinobacterial strains also present an alternative source of new molecules with strong bioactivities against multidrug-resistant *A. baumannii* strains.

Keywords *Acinetobacter baumannii* · Antibiotic · Antibiotic resistance · Actinobacteria · Antagonism

1 Introduction

Bacterial species belonging to the genus *Acinetobacter*, particularly *Acinetobacter baumannii*, have received increasing attention in recent years because of their potential to cause serious infections and for their ability to rapidly develop resistance to antibiotics. In fact, in the space of 40 years, this species have gone from being an “uninteresting bacterium” because it was not very pathogenic and happened to be sensitive to most of the antibiotics available at that time, to a pathogenic bacterium easily capable of acquiring multi-resistance to antibiotics [1].

The majority of infections caused by *A. baumannii* are contracted in hospitals, most often in critically ill patients hospitalized in intensive care or surgery [2]. In the 1970s, hospital-acquired pneumonia was the most common infection caused by this bacterium. Peleg et al. [3] reported that

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infections involving the central nervous system, skin, bone and soft tissues have appeared to be very problematic for some institutions.

The ability of this opportunistic pathogen to survive in the environment under adverse conditions for prolonged periods may have contributed to its endemic and epidemic behavior. This is also attributed to a few important factors which include the resistance of this agent to disinfectants, desiccation, nutritional deprivation in the moist environment and biofilm formation [4]. In addition, the persistent presence of *A. baumannii* in hospitals causes it to come into contact with antibiotics which exert a selective pressure favoring strains with antibiotic resistance [5].

In view of antibacterial resistance, the current work is directed toward the search for new antibiotics. *Actinobacteria* phylum occupies a prominent place among the microorganisms of interest in various fields, particularly in the medical field [6, 7]. They are able to secrete many bioactive molecules, the most important of which are antibiotics [8]. Our study aims to characterize the antibiotic resistance mechanisms in *A. baumannii* strains isolated at the Béni-Messous hospital (Algiers, Algeria) and to analyze the antagonistic activity of actinobacterial isolates against these pathogenic strains.

2 Materials and Methods

2.1 Sample Collection

Over the course of this study (1st February–30th May, 2018), a total of 664 samples were collected from patients in different services (reanimation, pediatrics and nephrology) and the outpatient department at the Béni Messous University Hospital Center. Specimens analyzed included blood (122), catheter (34), pus (198) and pulmonary (310).

2.2 Isolation and Identification of *Acinetobacter baumannii* Strains

All collected samples were cultured on blood and chocolate agar by the 4-quadrant streak plate method [9]. After 18 h of incubation (at 35 °C), pure cultures of presumptive *A. baumannii* were first subjected to the standard bacteriological techniques (morphology, Gram stain, catalase test and oxidase test, and MEVAG medium used to determine the pathway of attack of glucose and to test the carbohydrates used by oxidative bacteria) and identified later as *A. baumannii* by commercial API 20NE multi-test system (BioMérieux, Vitek, Marcy l'Etoile France) [10–12]. This biotyping method is a standardized system for the identification of non-fastidious, non-enteric and non-fermentative Gram-negative rods, combining eight

conventional tests which include potassium nitrate (NO₃), L-tryptophane (TRP), D-glucose (GLU), L-arginine (ADH), urea (URE), esculin ferric citrate (ESC), gelatin (GEL) and 4-nitrophenyl-β-D-galactopyranoside (PNPG) and also assimilation of 12 compounds, which include D-glucose (GLU), L-arabinose (ARA), D-mannose (MNE), D-mannitol (MAN), N-acetyl-glucosamine (NAG), D-maltose (MAL), potassium gluconate (GNT), capric acid (CAP), adipic acid (ADI), malic acid (MLT), trisodium citrate (CIT) and phenylacetic acid (PAC). Wells of biochemical test were inoculated with 0.5 McFarland bacterial suspension and incubated for 48 h (at 37 °C). The results were read after addition of appropriate reagents, as 7-digit number that identify by API 20NE analytical index.

2.3 Antibacterial Resistance Study

Antibacterial resistance of *A. baumannii* strains was determined for 11 antibiotics by the disk diffusion method. Sensitivity or resistance of each strain to the antibacterial drugs was determined according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI) [13]. Antibiotics tested were ticarcillin (75 µg), piperacillin (100 µg), ticarcillin + clavulanic acid (75/10 µg), ceftazidime (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (cotrimoxazole: 1.25/23.75 µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg) and doxycycline (30 µg).

Minimum inhibitory concentration (MIC) of colistin (polymyxin E) was determined by agar dilution method according to the recommendations of CLSI. Briefly, Mueller-Hinton (MH) agar containing different concentrations of colistin which ranged from 0.125 to 256 µg/ml was inoculated with a bacterial suspension whose turbidity was adjusted to 0.5 McFarland.

2.4 Antagonistic Activity of *Actinobacteria* Against *A. baumannii*

A total of 59 actinobacterial isolates were tested for antagonistic activity against the 19 *A. baumannii* strains using the agar plug diffusion method. The actinobacterial isolates were provided by the Laboratory of Biology of Microbial Systems of École Normale Supérieure of Kouba (Algiers, Algeria). They were isolated from Algerian soils and sediments. These Actinobacteria belonged to *Saccharothrix* and other closely related genera (13 isolates), *Actinomadura* (27 isolates) and *Streptomyces* (19 isolates). An agar culture of the Actinobacteria was made on ISP2 medium (yeast extract: 4 g, malt extract: 10 g, glucose: 4 g, distilled water: 1 L, pH: 7.2) by tight streaks on the plate surface and incubated for 10 days (at 30 °C). The agar cylinders (10 mm in diameter) were cut aseptically with a sterile cork borer from these Acti-

nobacteria cultures and deposited on the agar surface of MH plates previously inoculated by *A. baumannii* (0.5 McFarland), then incubated for 18 h (at 35 °C). After incubation, the antibacterial activity was evaluated by the measuring of the inhibition zone around the agar [14].

2.5 Testing for the ESBL Production

ESBL's production was tested by the Double Disk Synergy Test (DDST), using a disk of ticarcillin + clavulanic acid along with ceftazidime (cephalosporin). The bacterial strains were cultured on MH agar plates, as recommended by CLSI. A disk which contained ticarcillin + clavulanic acid (75/10 µg) was placed on the plate at a distance of 25 mm from that of ceftazidime (30 µg) and allowed to diffuse at room temperature (for 1 h). Once the time had elapsed, the disk of ticarcillin + clavulanic acid was replaced by another disk of ceftazidime. The plate was incubated for 18–24 h (at 35 °C). An increase greater or equal to 5 mm in the inhibition diameter of the ceftazidime disk applied after pre-diffusion of ticarcillin + clavulanic acid in comparison with the ceftazidime disk alone, was considered as positive for ESBL production [13].

2.6 Cephalosporinase Detection

All the bacterial strains were tested for cephalosporinase production by cloxacillin test. A suspension of 0.5 McFarland was prepared and inoculated on a MH agar plate, supplemented with cloxacillin (500 mg). The disks of ceftazidime (30 µg), tobramycin (10 µg), piperacillin (100 µg), ticarcillin (75 µg), cefepime (30 µg) and imipenem (10 µg) were placed on the inoculated surface of the agar. After 18 h of incubation (at 35 °C), the plates were examined for an increase in the diameter of the zone of inhibition around the disk of ceftazidime which indicated enzyme inactivation (positive result), as compared to the ceftazidime disk on the MH agar plate without cloxacillin [13].

2.7 Testing for Metallo- β -carbapenemase Production

EDTA test was used for the phenotypic detection of metallo- β -carbapenemase. Two 10 µg imipenem disks were placed on MH agar, and 4 µl of EDTA (ethylenediaminetetraacetic acid) solution was added to one of the disks. The inhibition zones of the imipenem disk and imipenem + EDTA disk were compared after 18 to 24 h of incubation (at 35 °C). An increased inhibition zone of the imipenem–EDTA disk compared to the imipenem disk alone was considered as MBL positive [13].

All the bacterial strains were also subjected to Hodge test for detection of metallo- β -carbapenemases. A 0.5 McFar-

land of *E. coli* ATCC 25922 suspension was prepared in 5 ml of sterile saline solution (0.9% NaCl, at room temperature), then 0.5 mm of the *Escherichia coli* ATCC 25922 suspension was added to 4.5 ml of saline solution to make a 1:10 dilution. Diluted suspension was inoculated on a MH plate by streaking over the entire plate with the help of a sterile cotton swab. A 10 µg imipenem disk was placed in the center of the MH plate. Radial streaks of the test organism, positive control (*A. baumannii* carbapenemase positive) and negative control (*E. coli* ATCC 25922), were done from the edge of the imipenem disk to the edge of the plate and then incubated (at 35 °C) in an ambient air for 18–24 h. Metallo- β -carbapenemases production was detected by the appearance of the enhanced *E. coli* ATCC 25922 growth along the test organism that revealed a clover-leaf-like indentation which indicated a positive test [10, 13].

3 Results and Discussion

3.1 Characteristics of *A. baumannii* Strains

Macroscopic examination of cultures showed smooth, circular, convex and whitish colonies. Microscopic examination after Gram differential staining revealed small bacilli or coccobacilli bacteria, with mixed (variable) staining (mainly pink and purple), rounded ends, isolated or grouped in two or in short chains. Enzymatic tests performed on the bacterial colonies presumptively identified as *Acinetobacter* (basing on morphological characterization and Gram stain), revealed a negative reaction to oxidase (oxidase -), positive reaction to catalase (catalase +) and oxidative degradation of sugars on MEVAG medium. All bacterial strains with the above characteristics were identified by API 20 NE (based on biochemical tests), which is widely used in hospital laboratories. After reading and interpretation of the API 20 NE strip, only 19 strains revealed very good identification level of *A. baumannii* with 7-digit number (0041072) according to the analytical profile index. Seven analytic profile index numbers that we have found (0041453, 000473, 0041173, 0041072, 0043073, 0045073, 0040053) have been reported for clinical and environmental *A. baumannii* strains [15]. Henein [16] reported two other analytic profiles: 0001073 and 1041073, which we also have detected.

3.2 Distribution of *A. baumannii* Strains According to the Nature of Samples

A total of 19 strains of *A. baumannii* were isolated from a variety of clinical samples as shown in Fig. 1. The obtained results indicated that a predominant number of *A. baumannii* strains were isolated from pulmonary samples (with a rate of 36.84%) and from the intensive care unit (57.89%).

Fig. 1 Distribution of *A. baumannii* strains according to the nature of samples

This observation was consistent with the results reported by Ben Haj Khalifa and Khedher [17] who stated the airway as the main isolation site for this species. Furthermore, Delbos [18] reported that hospital-acquired pneumonia is still the most common infection caused by *A. baumannii*. Baranzelli et al. [19] showed that the incidence of lower respiratory tract infections related to *Stenotrophomonas maltophilia* and *A. baumannii* is increasing, especially in intensive care units. These two Gram-negative bacilli are mainly identified in nosocomial infections such as ventilator-associated pneumonia, bacteremia and surgical wound infection. Between 2017 and 2019, several authors have shown the impact of *A. baumannii* in pulmonary infections. Tan et al. [20] reported that bacteremic pneumonia caused by *A. baumannii* has a higher mortality compared to other microorganisms. Wong et al. [21] reported that in Asian and certain Latin American countries, *A. baumannii* is one of the three most common causes of bacteremia and nosocomial pneumonia and in one well-publicized case, a health care worker developed fulminant pneumonia after inhalation of *A. baumannii* aerosolized during endotracheal suctioning of a ventilated patient. Huang et al. [22] reported that *A. baumannii* has been considered to be a prevailing pathogen causing ventilator-associated pneumonia (VAP), leading to high morbidity and mortality among patients in several intensive care unit (ICUs). In a retrospective study, these authors assessed clinical characteristics and antimicrobial resistance of *A. baumannii* in VAP on the basis of the patients between the year of 2015 and 2017. They found that the incidence of *A. baumannii*—VAP was approximately 35.9% in all VAP patients. Finally, from the molecular point of view, Martínez-Gutián et al. [23] showed that the *hisF* gene from *A. baumannii* ATCC 17978, found as over-expressed during a murine pneumonia infection, is involved in virulence, which places it as a new potential target for antimicrobial therapies.

Our results showed also that the presence of *A. baumannii* was involved in 31.58% of pus samples. This type of sample came from hospitalized patients who had undergone surgery. A similar rate (32.5%) was obtained by Falagas et al. [24]. However, a study carried out by Sileem et al. [25] revealed a

Table 1 Distribution of *A. baumannii* strains according to the provenance of samples

Provenance of samples	Number of <i>A. baumannii</i> strains	Rate of <i>A. baumannii</i> (%)
Hospital	Medical reanimation	11 78.94
	Pediatric department	2
	Nephrology department	2
Extern		4 21.05
Total		19 100

rate of 3.8% which was lower than that found in our study. *A. baumannii* strains were also isolated from blood and catheter samples, each with a rate of 15.79%. Research done by Uwingabiye et al. [26] indicated the presence of *A. baumannii* in 14.51% of blood samples, but according to Cisneros and Rodriguez Bano [27], *A. baumannii* was involved in only 1.3% of septicemia (rate recorded for a period of 7 years: from 1995 to 2002). Catheters colonized by *A. baumannii* were reported with a low rate of 3.85% by Uwingabiye et al. [26].

3.3 Distribution of *A. baumannii* Strains According to Origin of Samples

A. baumannii strains were isolated from different samples, especially those received from hospitalized patients with a frequency of 72.7% (15). Four strains (21.05%) came from outpatients who had been recently hospitalized, confirming the nosocomial nature of *A. baumannii* infections as reported by Bennett et al. [28] and Almasaudi [29].

Among the strains isolated from hospitalized patients, the medical reanimation department occupied first position with 57.89% of isolated strains, followed by the nephrology and the pediatric departments, each with a percentage of 10.52% (Table 1). A study carried out in Casablanca from 2001 to 2003, showed that 50% of *A. baumannii* strains came from the intensive care units [30].

3.4 Antibiotic Resistance Profile of *Acinetobacter baumannii* Strains

The antibiotic resistance profile of *A. baumannii* strains was determined using the disk diffusion technique as shown in Fig. 2.

Some strains were total resistant to all the 11 antibiotics tested while others were highly resistant (Table 2). Our results showed that all *A. baumannii* strains were resistant to all β -lactams, except for 19.09% strains that were sensitive to imipenem. However, lower resistance rates were recorded in Tunisia in 2009, where resistance to ticarcillin, piperacillin

Fig. 2 Antibigram result of *A. baumannii* strain S439. *AK* amikacin, *TM* tobramycin, *CIP* ciprofloxacin, *GN* gentamicin, *DO* doxycycline, *SXT* cotrimoxazole, *IMP* imipenem, *TIC* ticarcillin + clavulanic acid, *CAZ* ceftazidime, *TIC* ticarcillin, *PIP* piperacillin, *CL* colistin

Table 2 Antibiotic resistance profile of *A. baumannii*

Code of <i>A. baumannii</i> strains	β -lactams					Aminosides			Cyclines	Sulfamides	Quinolones	Polypeptides
	TIC	TCC	PIP	CAZ	IMP	AK	GN	TM	DO	SXT	CIP	CL
S1804, S439, S292 S483, S608, S460, S457, S54, S341, S214, S361 and S983	R	R	R	R	R	R	R	R	R	R	R	S
S1816	R	R	R	R	R	R	R	R	R	S	R	S
S344	R	R	R	R	S	S	S	R	R	S	S	S
S136	R	R	R	R	S	S	R	R	S	S	R	S
S568	R	R	R	R	S	S	S	R	S	S	S	S
S581	R	R	R	R	S	S	S	R	R	S	S	S
S01 and S1000	R	R	R	R	R	R	R	R	R	S	R	S

β -lactams: (*TIC* ticarcillin, *TCC* ticarcillin + clavulanic acid, *PIP* piperacillin, *CAZ* ceftazidime, *IMP* imipenem), aminosides: (*AK* amikacin, *GN* gentamicin, *TM* tobramycin), *DO* doxycycline, *SXT* cotrimoxazole, *CIP* ciprofloxacin, *CL* colistin, *R* resistant, *S* sensible

and ceftazidime was 51.7, 58.4 and 52.2%, respectively [17]. Grati et al. [31] reported the imipenem resistance rate as 87.5% which is close to our results (81.81%). In our study, the resistance of *A. baumannii* to aminoglycosides was recorded as 100% for tobramycin and exceeded 80% for gentamicin. These resistance rates were high compared to those found in Europe, which were 47.1% for gentamicin [32], and 41% for tobramycin [33]. Concerning the resistance to ciprofloxacin, a rate of 86.36% was noted in our study, which was close to that recently stated in Morocco (91%) [34]. These results allowed us to consider our strains as MDR (multidrug resistant) [35]. Lastly, all strains were sensitive to colistin with MICs not exceeding 1 μ g/ml. Our results were similar to those obtained by Kandeel [36], who stated the MIC of colistin as less than 2 μ g/ml.

3.5 Phenotypes of Antibiotic Resistance Mechanisms Characterized in *A. baumannii*

The antibiogram complimentary tests phenotypically revealed three enzymatic mechanisms of resistance in the tested *A. baumannii* strains: extended-spectrum β -lactamases (ESBL), cephalosporinase and metallo- β -carbapenemases as shown in Table 3.

Only three strains (15.79%), out of 19 tested, were ESBL producers. According to the national surveillance for resistant bacteria done in Algeria in 2009, the production of ESBL was 22.02% in 16 tested laboratories which show that the number of *A. baumannii* ESBL producers remains low [37]. Furthermore, screening of these strains for cephalosporinases revealed that 10 strains (52.63%) of *A. baumannii* were cephalosporinase positive. Yongrui and Xiangqun [38] revealed a higher rate (72%). The phenotypic tests EDTA and Hodge highlighted the production of metallo- β -carbapenemases by 15 strains (78.95%), similar to a rate that was found by Kaur et al. [39] (80.3%). On the other hand,

Table 3 Resistance phenotypes of *A. baumannii*

Code of <i>A. baumannii</i> strains	ESBL	Metallo- β -carbapenemase	Cephalosporinase
S54 and S893	+	+	+
S01, S214, S292, S341, S344, S439, S457, S460 and S1000	–	+	–
S136, S361, S483 and S581	–	+	+
S568, S608 and S1804	–	–	+
S1816	+	–	+

+ Present, – absent

lower rates (between 22 and 49%) of *A. baumannii* metallo- β -lactamase positive strains were reported by many authors [40–42].

3.6 Evaluation of the Antibacterial Potential of Actinobacterial Isolates

Fifty-nine actinobacterial isolates cultured on ISP2 medium were tested against 19 *A. baumannii* strains by agar plug diffusion method to evaluate their antibacterial potential. The antibacterial activity screening revealed that 19 (32.20%) isolates were active against at least one *A. baumannii* strain. In contrast, 40 (67.80%) isolates showed no activity. An antibacterial activity was obtained by all 13 *Saccharothrix* (or *Saccharothrix*-like) isolates, followed by five *Actinomadura* isolates and one isolate of *Streptomyces*. The results of these active isolates are recorded in Table 4.

The antagonistic activity against *A. baumannii* obtained in the 19 active isolates was generally strong for the majority of *Saccharothrix* (or *Saccharothrix*-like) isolates and the single *Streptomyces* isolate (WAB9). It was average for the five *Actinomadura* isolates. The diameters of inhibitions obtained by these active isolates varied between 10 and 30 mm. We noticed a variation in diameters of inhibition between isolates of the same genus; those of *Saccharothrix* (or *Saccharothrix*-like) ranged from 11 to 30 mm, whereas those of *Actinomadura* ranged from 10 to 22 mm.

The *Saccharothrix* isolates were highly active with a maximum inhibition diameter of 30 mm against some *A. baumannii* strains. In contrast, in the scientific literature, most antibiotics secreted by *Saccharothrix* isolates mainly target Gram-positive bacteria and fungi, and are rarely directed against Gram-negative bacteria [43–47].

Secondly, the *Streptomyces* sp. WAB9 followed with a maximum inhibition zone of 25 mm. Our results were similar to those of Yekkour et al. [48], who stated that this strain of *Streptomyces* sp. has a broad spectrum that extends over several filamentous fungi, and a large number of pathogenic bacteria (Gram-positive and Gram-negative), which are multi-resistant to several antibiotics. These authors found that the high activity of *Streptomyces* WAB9 (close to the species *Streptomyces ambofaciens*) is due to the production of a new antibiotic (named W9), which is a hydroxamic acid-containing molecule. Hydroxamic acid-containing molecules are known to exhibit low toxicities in general, which is very interesting.

Finally, *Actinomadura* was active with a maximum inhibition zone of 22 mm. *Actinomadura* isolates are known for the production of several antibacterial molecules, namely maduramicin, actinotiocin and carminomycin secreted, respectively, by *A. rubra*, *A. pusilla* and *A. carminata* [49].

The action spectrum of the antibacterial molecules secreted by the active isolates was quite broad for the majority of isolates with a maximal spectrum obtained by *Streptomyces* sp. WAB9.

4 Conclusion

During the course of this study, nineteen *A. baumannii* strains were obtained from various clinical samples at Béni Messous University Hospital Center (Algiers, Algeria). The majority of the strains were isolated from pulmonary samples received from patients hospitalized in the intensive care unit. The evaluation of antibiotic resistance of these strains against antibiotics recommended for treatment of *A. baumannii* infections revealed the ineffectiveness of the majority of the molecules. We noted a total absence of β -lactam antibacterial activity against all the strains. This was attributed to the production of different enzymes that degraded the antibiotic molecules namely metallo- β -carbapenemases and cephalosporinases. Nonetheless, all the *A. baumannii* strains were sensitive to colistin which was active at very low MICs. The results from the antagonistic potential evaluation carried out on a collection of Actinobacteria showed the ability of certain isolates belonging to the genera *Saccharothrix*, *Streptomyces* and *Actinomadura* to secrete bioactive molecules against *A. baumannii*. Thus, faced with this *A. baumannii* antibacterial resistance difficulty and a limited number of active molecules against it, Actinobacteria could be an alternative source of bioactive molecules that could meet this increased necessity.

Table 4 Antibacterial activity of actinobacterial isolates against *A. baumannii* strains

Actinobacterial isolates		Inhibition zone (mm) ^c																		
		<i>A. baumannii</i> strains																		
Code	<i>Saccharothrix</i> ^a	S1000	S54	S344	S457	S483	S439	S214	S581	S292	S568	S1816	S1804	S608	S01	S460	S136	S341	S361	S893
SA.al	<i>Saccharothrix</i> ^b	15	25	20	25	23	–	–	20	12	–	18	–	20	–	–	–	–	–	21
GA1	<i>Saccharothrix</i> ^b	18	24	20	22	23	–	24	–	28	20	–	–	26	–	26	24	25	–	25
GA2	<i>Saccharothrix</i> ^b	23	25	24	26	25	–	25	–	22	18	–	–	–	–	–	20	–	–	21
GA3	<i>Saccharothrix</i> ^b	20	26	22	25	25	–	26	–	20	20	–	13	14	–	28	18	13	15	20
GA4	<i>Saccharothrix</i> ^b	22	23	22	24	22	–	24	–	23	24	–	14	15	11	23	20	13	14	20
CA1	<i>Saccharothrix</i> ^b	–	24	–	14	20	–	20	20	–	–	27	25	26	–	23	30	23	–	25
CA2	<i>Saccharothrix</i> ^b	–	22	20	20	22	–	22	21	–	–	30	20	27	–	25	30	25	–	28
CA3	<i>Saccharothrix</i> ^b	–	22	21	23	22	–	–	–	–	–	20	–	23	–	–	–	–	–	20
CA4	<i>Saccharothrix</i> ^b	–	23	23	20	20	–	–	–	20	–	24	–	15	11	13	14	14	13	15
CA6	<i>Saccharothrix</i> ^b	–	–	–	–	–	–	–	–	20	11	25	15	16	14	15	14	20	16	16
CA9	<i>Saccharothrix</i> ^b	25	25	25	22	25	–	28	22	28	23	–	–	14	–	25	13	24	12	14
CA10	<i>Saccharothrix</i> ^b	23	25	25	22	25	–	25	25	30	25	–	20	25	–	25	24	27	–	25
TAM13	<i>Streptomyces</i>	17	19	17	19	18	18	19	19	10	11	–	–	–	–	–	–	–	–	–
WAB9	<i>Actinomadura</i>	13	15	15	14	14	12	12	14	12	15	15	13	13	12	12	–	15	25	14
BKS2	<i>Actinomadura</i>	–	–	–	–	–	–	–	–	15	16	15	18	15	–	–	–	–	–	–
AHO1	<i>Actinomadura</i>	–	–	–	–	–	–	–	–	20	21	22	16	20	22	22	20	18	20	21
AHO10	<i>Actinomadura</i>	–	–	–	–	–	–	15	18	15	18	11	17	16	15	21	20	20	20	18
BAS26	<i>Actinomadura</i>	–	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ETM13	<i>Saccharothrix</i> ^a	15	22	20	15	15	15	–	15	–	15	–	–	–	–	–	–	–	18	16

– Absence of activity. Forty other strains of actinobacteria showed no antagonistic activity against the target microorganisms

^aSaccharothrix algeriensis DSM 44581^bSaccharothrix or Saccharothrix-like isolates^cThe diameter of the cylinder (10 mm) is not included in the values

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