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Contribution to the study of biofilm production of *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* isolated from Tamanrasset hospital and biofilm biological control assay

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"المساهمة في دراسة إنتاج الأغشية الحيوية لبكتيريا *العنقودية الذهبية و الزائفة الزنجارية و الاشريكية القولونية* المعزولة من مستشفى تمنراست وفحص المكافحة البيولوجية للأغشية الحيوية."

الملخص:

لعدة سنوات، كانت الأغشية الحيوية موضوعًا للعديد من الأبحاث في مجالات مختلفة. تشكل بعض أنواع العدوى المرتبطة بالأغشية الحيوية الميكروبية مصدرًا للقلق في القطاع الطبي. وفي هذا الصدد، فإن البيانات العلمية قليلة أو غير متوفرة في المؤسسات الصحية في الجزائر. وفي هذا السياق ركز هدف هذه الدراسة على دراسة السلالات البكتيرية المعزولة من العينات البيولوجية المأخوذة من المرضى الموجودين في مستشفى مصباح بغداد، تمنراست، الجزائر. خصص الجزء الأول للدراسة البكتريولوجية للسلالات السريرية للمكورات العنقودية الذهبية والزائفة الزنجارية والإشريكية القولونية وقدرتها على تكوين الأغشية الحيوية. أما الجزء الثاني فقد ركز على مكافحة هذه الأغشية الحيوية باستخدام البكتيريا الأكتينية المعزولة من التربة في أقصى جنوب الجزائر بولاية تمنراست.

تم إجراء تحديد السلالات السريرية بالطرق الميكروبيولوجية التقليدية ثم تم تأكيدها بواسطة تقنية MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry). تم إجراء مخطط المضادات الحيوية باستخدام النظام الآلي BD Phoenix ™ (Becton Dickinson، الولايات المتحدة الأمريكية). من بين 30 عزلة سريرية، تم الاحتفاظ بـ 9 (45%) سلالات من المكورات العنقودية الذهبية، 2 (10%) سلالات من الزائفة الزنجارية و9 (45%) سلالات من الإشريكية القولونية. جميع سلالات المكورات العنقودية الذهبية هي منتجة لـ β-lactamase (BLACT)، اثنان منها، السلالتان 01 و02، مقاومتان للميثيسيلين (MRSA). سلالتان من *E. coli* ، 04 و09 منتجتان لفئة كاربابينيماز (CARBD) من الفئة D وبيتا لاكتاماز ممتد الطيف (ESBL)، على التوالي. وبالإضافة إلى ذلك، فإن السلالة 09 متعددة المقاومة (MDR-*E. coli*).

تم العثور على 10%، 30%، و50% من العزلات السريرية عالية، متوسطة، ومنخفضة في إنتاج الأغشية الحيوية، على التوالي، من خلال الطريقة المظهرية لأجار الكونغو الأحمر (CRA). كشف التقييم النوعي للأغشية الحيوية باستخدام طريقة الالتصاق بالأنبوب (TAM) أن سلالات E. coli و*P. aeruginosa* منتجة للأغشية الحيوية بشكل كبير مقارنةً بسلالات *S. aureus*. علاوة على ذلك، وفقًا لطريقة التخفيف الدقيق على صفيحة ميكروية ذات 96 بئرًا (MTP: Microtiter Plate)، فإن غالبية السلالات السريرية لديها إنتاج معتدل للأغشية الحيوية (0.4< OD_{630nm} ≤0.8). كانت سلالات *S. aureus* (01، 02، 09)، وسلالات *E. coli* (06، 08)، هي الأكثر إنتاجية للأغشية الحيوية على التوالي (OD_{630nm} =0.75، 0.74، 0.73 و0.74، 0.74). في حين أن السلالات الأخرى لديها في المتوسط OD_{630nm} بين 0.49 و 0.69 باستثناء سلالة المكورات العنقودية الذهبية 05 والتي كانت عبارة عن غشاء حيوي ضعيف (OD_{630nm} = 0.33).

كانت الأكتينوباكتريا هي العوامل المختارة للتحكم البيولوجي في الأغشية الحيوية. تم عزلهم من سبعة تربة ريزوسفيرية من السنط من سبعة مواقع باستخدام الزراعة المعتمدة على أجار مستخلص الخميرة والجلسرين (GYE). في الواقع، تم الحصول على 124 سلالة، ويبدو أن توصيفها العياني والمجهري يتوافق مع سلالات الأكتينوباكتريا مع التنوع في اتساقها العياني. كشف التحديد الجزيئي 16s rRNA من 24 بكتيريا شعوية أن السلالات تنتمي إلى خمسة أجناس مختلفة ذات تشابه منخفض أو أكبر من 98.6٪؛ بما في ذلك الستربتوميسيس، والنوكارديوبسيس، والميكرومونوسبورا، والأكتينومادورا، والسيلولومواناس. بينما على مستوى الأنواع، هناك ستة سلالات فقط من البكتيريا الأكتينوبكتريا D4 و D14 و D25 و D31 و D33 و D40 قريبة من الأنواع Streptomyces lomondensis|NBRC 15426|AB184673، Streptomyces tuirus|NBRC 15617|AB184690، Streptomyces bellus|ISP 5185|AJ399476T و Streptomyces longhuiensis|MW680654T| BH-MK-02.

كشفت طريقة التضاد المتقاطع أن غالبية عزلات البكتيريا الأكتينوبكتريا كانت نشطة للغاية ضد جميع سلالات المكورات العنقودية الذهبية (06 مم ≤ منطقة التثبيط ≤ 35 مم)، وأظهرت سلالات البكتيريا الأكتينوبكتريا D25، D32 نشاطًا انتقائيًا ضد جميع سلالات *S. aureus*. مع متوسط منطقة تثبيط (مم) تتراوح [من 4 إلى 20] و [من 2 إلى 8] على التوالي. مع الإشريكية القولونية من 2 إلى 25 ملم، حيث تمتلك سلالات الأكتينوباكتريا D24 و D42 نشاطًا انتقائيًا فقط ضد سلالات *E. coli* (منطقة التثبيط [6-20]، [5-11] على التوالي). بينما أظهرت سلالات *P. aeruginosa* مقاومة قوية لسلالات الأكتينوباكتريا. ومع ذلك، فإن سلالات الأكتينوباكتريا D31، D33، D35، D36 و D47 لها أكبر طيف من التأثيرات على البكتيريا السريرية المعزولة، حيث كان النشاط المضاد [3-6]، [2-5]، [20 -35]، [20 -34] و[7-15] على التوالي ضد جميع سلالات *S. aureus* ، وكذلك ضد جميع سلالات الإشريكية القولونية [5-11]، [2-7]، [18-25]، [4-6]، [4-20].

تعتبر السلالة D35 من أهم العزلات من حيث نشاطها العدائي. بالإضافة إلى ذلك، كشف اختبار المكافحة الحيوية للمستخلص الخام (EX) ضد السلالات السريرية أن غالبية المستخلص الخام له تأثير متضائل بشكل كبير على إنتاج الأغشية الحيوية. في حين كانت أفضل درجة نشاط مضاد للأغشية الحيوية هي EX104 (من D16) ضد سلالات *S. aureus* 09 و10 (من OD_{630nm} = 0.73 إلى OD_{630nm} = 0.30)، يليه Ex115 (من D47) ضد *E. coli* 02 (من OD_{630nm} = 0.53 إلى OD_{630nm} = 0.26). وفي الوقت نفسه، قام EX 104 بتنفيذ ست سلالات من *E. coli*: 01، 04، 06، 07، 08 و10، أفضل النتائج كانت ضد *E. coli* 08 (من OD_{630nm} = 0.74 إلى OD_{630nm} = 0.52). في حين أن الأغشية الحيوية لسلالات *P. aeruginosa* 01 و09 تأثرت بالمستخلص الخام EX27 (من D35) (من OD_{630nm} = 0.66 إلى OD_{630nm} = 0.38).

كلمات مفتاحية: المكورات الذهبية العنقودية، الاشريكية القولونية، الزائفة الزنجارية، الاغشية الحيوية، الاكتينوبكتريا، التحكم الحيوي.

« Contribution to the study of biofilm production of *Staphylococcus aureus.*, *Pseudomonas aeruginosa* and *Escherichia coli* isolated from Tamanrasset hospital and biofilm biological control assay»

Abstract:

For several years, biofilms have been the subject of many researches in different fields. Certain infections associated with microbial biofilms are a source of concern in the medical sector. In this regard, scientific data are few or not available in health institutions in Algeria. It is in this context that the objective of this study focused on the study of bacterial strains isolated from biological samples taken from patients hospitalized at the MESBAH BAGHDAD Hospital, Tamanrasset, Algeria. The first part was devoted to the bacteriological study of clinical strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* and their ability to form biofilm. The second part focused on the fight against these biofilms using Actinobacteria isolated from the soil in the extreme south of Algeria, Tamanrasset.

The identification of clinical strains was carried out by conventional microbiological methods and then confirmed by the MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry) technique. The

antibiogram was performed using the BD Phoenix™ automated system (Becton Dickinson, USA). Among the 30 clinical isolates, 9 (45%) strains of *Staphylococcus aureus*, 2(10%) strains of *Pseudomonas aeruginosa* and 9(45%) strains of *Escherichia coli* were retained. All S. aureus strains are β-lactamase (BLACT) producers, two of them, strains 01 and 02, are resistant to methicillin (MRSA). Two strains of *E. coli*, 04 and 09 are producers of class D carbapenemase (CARBD) and extended spectrum beta-lactamase (ESBL), respectively. In addition, strain 09 is multi-resistant (MDR-*E. coli*).

10%, 30%, and 50% of clinical isolates were found to be highly, moderately, and poorly biofilm-producing, respectively, by the Congo Red Agar (CRA) phenotypic method. Qualitative assessment of the biofilm by the tube adherence method (TAM) revealed that the strains of *E. coli* and *P. aeruginosa* are highly biofilm-producing as opposed to *S. aureus* strains. Furthermore, according to the microdilution method on 96-well microplate (MTP: Microtiter Plate), the majority of clinical strains have moderate biofilm production (0.4< OD630nm ≤0.8). The strains of *S. aureus* (01, 02, 09), and the strains of *E. coli* (06, 08), were respectively the most productive of biofilm (OD630nm = 0.75, 0.74, 0.73 and 0 .74, 0.74), while the other strains have on average an OD630nm between 0.49 and 0.69 except *S. aureus* strain 05 which was a weak biofilm former (OD630nm = 0.33).

Actinobacteria were the selected agents for the biological control of biofilm. They were isolated from seven acacia rhizospheric soils from seven sites using dependent culture on glycerol yeast extract (GYE) agar. In fact, 124 strains were obtained, their macroscopic and microscopic characterization nevertheless seems to correspond to Actinobacteria strains with diversity in their macroscopic consistency. Molecular identification of 16s rRNA from 24 Actinobacteria revealed that the strains belonged to five different genera with low or greater than 98.6% similarity; including Streptomyces, Nocardiopsis, Micromonospora, Actinomadura, Cellulomonas. While at the species level, only six strains of Actinobacteria D4, D14, D25, D31, D33 and D40 are close to the species *Streptomyces lomondensis*|NBRC 15426|AB184673, *Streptomyces tuirus*|NBRC 15617|AB184690, *Streptomyces bellus*|ISP 5185|AJ399476^{T} and *Streptomyces longhuiensis*|BH-MK-02|MW680654^{T}.

The cross-streak antagonism method revealed that the majority of Actinobacteria isolates were highly active against all *S. aureus* strains (06 mm ≤ zone of inhibition ≤ 35 mm), Actinobacteria strains D25, D32 , showed selective activity against all strains of *S. aureus* with an average inhibition zone (mm) varied [from 4 to 20] and [from 2 to 8] respectively. With *E. coli* from 2 to 25 mm, where Actinobacteria strains D24 and D42 have selective activity only against *E. coli* strains (inhibition zone [6-20], [5-11] respectively). While *P. aeruginosa* strains showed strong resistance to Actinobacteria strains. However, Actinobacteria strains D31, D33, D35, D36 and D47 have the greatest spectrum of effects on isolated clinical bacteria, where the antagonistic activity was [3-6], [2-5], [20 -35], [20 -34] and [7-15] respectively against all strains of *S. aureus*, as well as against all strains of *E. coli* [5-11], [2-7], [18-25], [4-6] and [4-20].

Strain D35 is the most important isolate regarding to its antagonistic activity. Additionally, biocontrol testing of the crude extract (EX) against clinical strains revealed that the majority of the crude extract has a significantly diminished effect on biofilm production. While the best anti-biofilm activity score was that of EX104 (from D16) against *S. aureus* strains 09 and 10 (from OD630nm = 0.73 to OD630nm = 0.30), followed by Ex115 (from D47) against *E. coli* 02 (from OD630nm = 0.53 to OD630nm = 0.26). Meanwhile, EX 104 carried out the six strains of E. coli: 01, 04, 06, 07, 08 and 10, the best results are against *E. coli* 08 (from OD630nm = 0.74 to OD630nm = 0.52). Whereas the biofilm of *P. aeruginosa* strains 01 and 09 was affected by crude extract EX27 (from D35) (from OD630nm = 0.66 to OD630nm = 0.38).

Key words*: Staphylococcus aureus, Escherichia coli, P. aeruginosa*, Biofilm, Actinobacteria, Biocontrol

« Contribution à l'étude de la production de biofilms de *Staphylococcus aureus*., *Pseudomonas aeruginosa* et *Escherichia coli* isolés à partir de l'hôpital de Tamanrasset et essayes dans des contrôle biologique des biofilms»

Résumé :

Depuis plusieurs années, les biofilms font l'objet de plusieurs recherches dans différents domaines. Certaines infections associées au biofilms microbiens sont une source d'inquiétude dans le secteur médical. A cet égard, les données scientifiques sont peu ou pas disponibles dans les institutions de santé en Algérie. C'est dans ce contexte que l'objectif de cette étude a porté sur l'étude des souches bactériennes isolées à partir des prélèvements biologiques effectués sur les patients hospitalisés à l'hôpital MESBAH BAGHDAD, Tamanrasset, Algérie. La première partie a été consacrée à l'étude

bactériologique des souches cliniques de *Staphylococcus aureus, Pseudomonas aeruginosa* et *Escherichia coli* et leur capacité à former le biofilm. La deuxième partie était focalisée sur la lutte contre ces biofilms par le biais des Actinobactéries isolées à partir du sol dans l'extrême sud en Algérie, Tamanrasset.

L'identification des souches cliniques a été réalisée par les méthodes microbiologiques conventionnelles puis confirmée par la technique MALDI-TOF MS (Matrix Assisted Laser Desorption Ionisation-Time Of Flight Mass Spectrometry). L'antibiogramme a été effectué à l'aide du système automatisé BD Phoenix™ (Becton Dickinson, USA). Parmi les 30 isolats cliniques, 9(45%) souches de *Staphylococcus aureus,* 2(10%) souches de *Pseudomonas aeruginosa* et 9(45%) souches de *Escherichia coli,* ont été retenues. Toutes les souches de *S. aureus* sont productrices de β-lactamase (BLACT), deux d'entre elles, la souche 01 et 02, sont résistantes à la méthicilline (MRSA). Deux souches d'*E. coli,* O4 et 09 sont productrices de carbapénémase de classe D (CARBD) et bêta- lactamase à spectre étendu (ESBL), respectivement. De plus la souche 09 est multirésistante (MDR-*E. coli*).

10%, 30% et 50% des isolats cliniques se sont avérées fortement, modérément et faiblement productrices de biofilm, respectivement, par la méthode phénotypique Congo Red Agar (CRA). L'évaluation qualitative du biofilm par la méthode des tubes (TAM : tube adherence method) a révélé que les souches d'*E. coli* et de *P. aeruginosa* sont fortement productrices de biofilm par opposition aux souches de *S. aureus*. Par ailleurs, selon la méthode de microdilution sur microplaque 96 puits (MTP : Microtiter Plate), la majorité des souches cliniques ont une production modérée de biofilm (0,4< OD_{630nm} ≤0,8). Les souches de *S. aureus* (01, 02, 09), et les souches d'*E coli* (06, 08), étaient respectivement les plus productrices de biofilm (OD_{630nm} = 0,75, 0,74, 0,73 et 0,74, 0,74), tandis que les autres souches ont en moyenne une OD_{630nm} comprise entre 0,49 et 0,69 à l'exception de la souche 05 de *S. aureus* qui était faiblement formatrice de biofilm (OD_{630nm} = 0,33).

Les Actinobactéries sélectionnées pour le contrôle biologique du biofilm. Ont été isolées à partir de sept sols rhizosphériques d'acacia provenant de sept sites en utilisant une culture dépendante sur gélose à l'extrait de levure au glycérol (GYE). En fait, 124 souches ont été obtenues, leur caractérisation macroscopique et microscopique semble néanmoins correspondre à des souches d'Actinobacteria avec une diversité dans leur consistance macroscopique. L'identification moléculaire de l'ARNr 16s de 24 Actinobactéries a révélé que les souches appartenaient à cinq genres différents avec une similarité faible ou supérieure à 98,6 % ; dont *Streptomyces*, *Nocardiopsis, Micromonospora, Actinomadura, Cellulomonas*. Alors qu'au niveau des espèces, seules six souches d'*Actinobacteria* D4, D14, D25, D31, D33 et D40 sont proches des espèces *Streptomyces lomondensis*|NBRC 15426|AB184673, *Streptomyces tuirus*|NBRC 15617|AB184690, *Streptomyces bellus*|ISP 5185|AJ399476T et *Streptomyces longhuiensis*|BH-MK-02|MW680654T.

La méthode d'antagonisme par stries croisées a révélé que la majorité des isolats d'Actinobacteria étaient extrêmement actifs contre toutes les souches de *S. aureus* (06 mm ≤ zone d'inhibition ≤ 35 mm), les souches d'Actinobacteria D25, D32, ont montré une activité sélective contre toutes les souches de *S. aureus* avec une moyenne de zone d'inhibition (mm) variée [de 4 à 20] et [de 2 à 8] respectivement. Avec *E. coli* de 2 à 25 mm, où les souches d'Actinobacteria D24 et D42 ont une activité sélective uniquement contre les souches d'*E. coli* (zone d'inhibition [6-20], [5-11] respectivement). Alors que les souches de *P. aeruginosa* présentaient une forte résistance aux souches d'Actinobacteria. Cependant, les souches d'Actinobacteria D31, D33, D35, D36 et D47 ont le plus grand spectre d'effets sur les bactéries cliniques isolées, où l'activité antagoniste était [3-6], [2-5], [20-35], [20 -34] et [7-15] respectivement contre toutes les souches de *S. aureus*, ainsi que contre toutes les souches d'E. coli [5-11], [2-7], [18-25], [4-6] et [4-20]. La souche D35 est l'isolat le plus important en ce qui concerne son activité antagoniste. De plus, les tests de biocontrôle de l'extrait brut (EX) contre des souches cliniques ont révélé que la majorité de l'extrait brut a un effet diminué de manière significative sur la production de biofilms. Alors que le meilleur score d'activité anti-biofilm était celui de l'EX104 (de D16) contre les souches 09 et 10 de *S. aureus* (de OD_{630nm} = 0,73 à OD_{630nm} = 0,30), suivi de l'Ex115 (de D47) contre *E. coli* 02 (de OD_{630nm} = 0,53 à OD_{630nm} = 0,26). Alors que, l'EX 104 a effectué les six souches d'*E. coli* : 01, 04, 06, 07, 08 et 10, les meilleurs résultats sont contre *E. coli* 08 (de OD630nm = 0,74 à OD630nm = 0,52). Tandis que le biofilm des souches 01 et 09 de *P. aeruginosa* était affecté par l'extrait brut EX27 (de D35) (de OD_{630nm} = 0,66 à OD_{630nm} = 0,38).

Mots clés: *Staphylococcus aureus, Escherichia coli, P. aeruginosa*, Biofilm, Actinobacteria, Biocontrol

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

«... يَرۡفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمۡ وَٱلَّذِينَ أُوتُواْ ٱلۡعِلۡمَ دَرَجَٰتٖۚ وَٱللَّهُ بِمَا تَعۡمَلُونَ خَبِيرٞ»

اية 11 سورة المجادلة

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Dedication

*Family **REGGANI** and **HAIBAOUI***

To my Dears Parents, sisters and brothers

Today is the day I have seen all your visions on, the day when I finally take one big leap forward from education to career. As I put on my graduation gown and hold my caps, memories of the sacrifices you both made for me to reach this day filled me with unbelieving intensity. I have always known them, but actually never thought or talked much about them until today. Looking at myself now, I know I would never have made it without you behind me.

*This work is for you, my dad **MOHAMMED**, my mother's **REGGANI MOHAMED AICHA**, and **HABIBA DRICI;** my sisters **ZOHRA**, **MOULATI** and **CHERIFA**; my brothers **ABDALLAH**, **AHMED**, **ZIN EDDIN** and **OMAR**. My little kittens: **RIYADH, KHAMESSE, SOUMOUD** and **ANNAH.***

That always you are happy for my happiness, my success and give me the hope when I feeling frustrated.

God bless you always.

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Aap	Accumulation-associated proteine
AIs	autoinducers
Agr	Accessory gene regulator
AgNPs	Argent nanoparticles
AMR	antimicrobial resistance
Atl	Major autolysin
ATP	Adenosine triphosphate
Bap	Biofilm associated protein
BH1CC	MRSA strain BH1CC
bp	base pair
BFRT	Biofilm Ring Test
C-di-GMP	Cyclic diguanylate
CMEB	Biofilm eradicating minimum concentration
CNDs	carbon nanodots
ECM	Mucilaginous extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EPM	Extracellular polymeric matrix
EPS	Extracellular polymeric substance, Extrapolymeric substance, exopolysaccharide
ESBL	Extended spectrum β-lactamases E.coli
exo	Exotoxins
ExPEC	Extra-intestinal pathogenic *E. Coli*
FESEM	field emission scanning electron microscopy
FnBPs	Fibronectin-binding proteins
Hla	Alpha-toxin
IL	Interleukins
IMDs	Indwelling medical devices
IS	Insertion sequence
ica	Intercellular adhesion
icaR	Gene of protein regulator of biofilm formation
LukAB	Leukocidin AB
M1	Proinflammatory microbicidal phenotype in macrophages
MDSCs	Myeloid derived suppressor cells
MDR	Multidrug-resistant
M-FISH	multiplex fluorescence in situ hybridization
MRSA	Methicillin-resistant S. Aureus
MSSA	Methicillin-susceptible S. aureus
NIH	National Institutes of Health
PGA	Poly-β-1,6-D-N-acetylglucosamine
PIA	Polysaccharide intercellular adhesion
pH	Potential hydrogen
PMN	Polymorphonuclear neutrophils
PNA	peptide nucleic acid
qRT-PCR	Real Time Quantitative-Reverse Transcription- PCR
QS	Quorum sensing
Rbf	Protein regulator of biofilm formation
SasG	S. Aureus surface protein g
SEM	Scanning electron microscopy
FESEM]	field emission SEM
SpA	S. aureus protein A

Spx	Strees protiens x Genes
sRNAs	Regulatory small rnas
SrrAB	Staphyloccocal respiratory response AB
TEM	Transmission electron microscopy
TcaR	Tiecoplanin-associated locus regulator
Th1/Th2	T helper cells type 1/2
Treg	Lymphocites T regulators
TSB	Tryptophane soya broth
UTI	Urinary tract infections
UV	Ultraviolet
VBNC	Viable non cultivable cells
VF	Virulence factors
WHO	World health organization

Introduction:

Microorganisms were the first form of life on our planet (Juhas, 2023), they bring together bacteria (prokaryotes), yeasts, algae, fungi and protozoa . These living beings are ubiquitous, colonizing soils (Jansson & Hofmockel, 2018), fresh and marine waters and atmosphere (Dai et al., 2021; Martiny et al., 2006), human and are associated with each other through relationships adapted to their biological needs (Dekaboruah et al., 2020; Pommerville, 2013). They are essential to the human and the environment by contributing to major cycles of matter and playing an essential role in almost all ecosystems (Maftei et al., 2024; Wang et al., 2024). Once that a surface is immersed in a fluid, it can be colonized by microorganisms and be covered with a biofilm (Dang & Lovell, 2016).

For several years, biofilms have been the target of important research, showing that their biological properties are diverse from the organisms that constitute them in their planktonic level (Bystrianský et al., 2019). Thus, there is a multitude of environments where they can developed. In fact, they have the ability to grow on any type of natural or artificial surface, whether mineral (rock, air-liquid interfaces, etc.), organic (skin, digestive tract of animals, roots and plant leaves), industrial (pipes, ship hulls) or medical (prostheses ,catheters, urinary catheters, etc.), it should be note that biofilm may or may not be pathogen (Herrling et al., 2019). This system called “biofilm” by Bill Costerton et al in 1978 (Costerton et al., 1978, 1999).

The bacteria biofilms communities can be formed from homogenic or heterogenic species that are immersed in an extracellular structural matrix, that protects them from harsh environment and immune system (S. Singh et al., 2021). Implantable medical devices, the transformation of planktonic cells into biofilm forming cells occurs in response to a variety of environmental stimuli, including nutrient availability, surface type, and so on Bacteria adhere to surfaces and undergo subsequent changes such as increased EPS secretion Proteins, polysaccharides, DNA, and other fibers of adhesion (LewisOscar et al., 2021).

Biofilms can be found on tissues that have long been known to harbor commensal microbiota, in some types of samples, biofilms primarily occur as aggregates suspended in mucus or other host secretions like pus or urine. In others, the biofilms are attached to the tissue itself typically at a mucosal interface (Perry & Tan, 2023).

Biofilm diseases affected the most tissues of human body: in the auditory, the cardiovascular, the digestive, the integumentary, the reproductive, the respiratory, and the urinary system (Vestby et al., 2020) they may cause local tissue damage and later cause a severe infection. The most frequency bacteria can be caused an associated biofilm infections are *Fusobacterium nucleatum, Klebsiella*

pneumoniae, Proteus mirabilis, Streptococcus viridans, Enterococcus faecalis, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli and *Staphylococcus aureus,* even that Gram positive or Gram negative bacteria (Khatoon et al., 2018; S. Sharma et al., 2023).

Biofilms have been found to be involved in a wide variety of microbial infections (by one estimate 80% of all infections). These include cystic fibrosis pneumonia, periodontal disease, dental caries, otitis media, musculoskeletal infections, necrotizing fascitis, biliary tract infection, osteomyelitis, bacterial prostatitis, native valve endocarditis, meloidosis, and peri-implantitis. Salient features of these infections are persistence and chronicity (Chandki et al., 2011; Gondil & Subhadra, 2023).

The properties of biofilm are not exclusive only to scuffle the harsh environment conditions, but also contributing in emerged of drug and multidrug resistance bacteria by the effect of the exchange of the flexible genetic material between the species (Michaelis & Grohmann, 2023).

Microorganisms in a biofilm are extremely higher resistant to antibiotics than in their planktonic state. The mechanisms of this increased resistance varied from species to another, antibiotic to another, and which environments are grow. This antibiotic resistance in bacteria is supposed to be influenced by their nutritional status, growth rate, temperature, pH and prior exposure to subeffective concentrations of antimicrobial agents. In the other hand, the slow rate of growth of bacterial species in a biofilm makes them less susceptible and the ECM less diffusible to bactericidal antibiotics (Chandki et al., 2011; Shree et al., 2023)

Another medical challenge is MDR biofilm-associated bacterial infections. In fact, The emergence of antibiotic resistance within a tolerant biofilm population could therefore constitute an aggravating factor increasing the frequency of therapeutic failure and infection recurrence (Karami et al., 2020). Biofilm-forming microorganisms are estimated to cause 65–80% of human infections (Sionov & Steinberg, 2022).

Biofilms indeed display a characteristic high level of tolerance to a broad range of antibiotics that disappears quickly after biofilm dispersion (Usui et al., 2023). the three-dimensional structure protects microbial communities from biotic and abiotic factors like toxic substances, predation, and other environmental stress (Gloag et al., 2020).

The biofilm's extracellular matrix serves as a protective barrier since a result, biofilm bacteria are more resistant to mechanical and chemical assaults than planktonic bacteria (Assefa & Amare, 2022). Additionally, biofilm offers a wide range of micro niches that support a substantially varied microfauna and metabolic potential, which present an opportunity for novel genotype (Coenye et al., 2022).

Because of all these traits, biofilms substantially increase bacterial antibiotic resistance virulence of pathogenic bacteria (Shree et al., 2023).

Therefore, the study of biofilms and the strategies to eliminate them is one of the most important fields of research in the present days, many reviews on anti-biofilm compounds already have been done (Gao et al., 2024; Shrestha et al., 2022; Silva et al., 2023). The identified anti-biofilm compounds are generally isolated from the natural sources, some synthetic compounds, chelating agents, and lantibiotics also have been found to possess anti-biofilm activity (Sayem et al. 2011; Bueno 2014; Leetanasaksakul and Thamchaipenet 2018). The different anti-biofilm molecules along with their target microorganisms, these anti-biofilm molecules follow different mechanisms to inhibit biofilm formation in different bacteria (Pinto et al., 2022).

Antibiotics are conceivably the most effective chemotherapy created in the 20th century and perhaps throughout medical history. Since their discovery over seven decades ago, antibiotics have saved countless lives daily (Hutchings et al., 2019). Antibiotics' effectiveness in treating and preventing infections is critical in modern medicine and also needed for common and complex medical operations, including C-sections and organ transplants (Benyamini, 2024; Shrestha et al., 2022). It has been demonstrated without a shadow of a doubt that bacteria might learn to resist the effects of antibiotic therapy, and the formation of biofilms is the fundamental cause of the issue (Urban-Chmiel et al., 2022).

For that reason, the discovery of compounds with antibacterial activities has paved the way to saving the lives of patients with serious infectious diseases. Research on microorganisms as potential sources of new and effective therapeutic agents with different modes of actions has been recognized, one of the attractive bio-resources of novel bioactive compound is Actinobacteria (Leetanasaksakul & Thamchaipenet, 2018), this later have been reported to produce various bioactive compounds of medical interests including antibacterial, antifungal, antiviral, anticancer, and neuroprotective agents (Azman et al., 2019).

Through this scientific research, we contribute to the study of biofilm formation ability from the medical side as an essential sector that concern human health, targeting three different clinical bacterial species: *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, which have a higher frequency in infectious diseases and biofilm-associated infections. Besides of that, we investigate their ability to forming single biofilm specie. On the other hand, as an assay on biological control we look for new antibacterial/anti-biofilm compounds produced by Actinobacteria as promising source of bioactive secondary metabolites.

CHAPTER 1*: REVEIU AND LETERATTURE*

I. BIOFILMS and generalities on Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli

I.1. Historie and definition:

Van Leeuwenhoek, using his simple microscopes, first observed microorganisms on tooth surfaces and can be credited with the discovery of microbial biofilms (Heukelekian & Heller, 1940). Zobell observed that the number of bacteria on surfaces was dramatically higher than in the surrounding medium (in this case, seawater) (Zobell, 1943). However, a detailed examination of biofilms would await the electron microscope, which allowed high-resolution photo-microscopy at much higher magnifications than did the light microscope. Jones et al. used scanning and transmission electron microscopy to examine biofilms on trickling filters in a wastewater treatment plant and showed them to be composed of a variety of organisms (based on cell morphology) (H. C. Jones et al., 1969).

Based on observations of dental plaque and sessile communities in mountain streams, Casterton et al in 1978 put forth a theory of biofilms that explained the mechanisms whereby microorganisms adhere to living and nonliving materials and the benefits accrued by this ecologic niche (Costerton et al., 1978). Since that time, the studies of biofilms in industrial and ecologic settings and in environments more relevant for public health have basically paralleled each other (O'Toole et al., 2000). Much of the work in the last 2 decades has relied on tools such as scanning electron microscopy (SEM) or standard microbiologic culture techniques for biofilm characterization (Characklis, 1973; Costerton et al., 1978). A biofilm is an aggregation of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material (Haque et al., 2021). cellular or non-cellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix (Zhao et al., 2023).

I.2. Microbial habitats in the human body:

Historically, the field of microbiome research emerged from environmental microbiome research and later evolved into viewing eukaryotes as inseparable from the microbial community with which they share space. After all, the human body is an ecosystem where trillions of tiny organisms coexist with the host (Dekaboruah et al., 2020). The scientific term "microbiome" therefore refers to the set of genes of all microorganisms that inhabit almost all human body parts. The microbiome is thus considered as a second genome that has a symbiotic relationship with the host. This relationship maybe positive or beneficial, negative or pathogenic, or neutral; hence, microbiome interactions play a key role in human health (Juhas, 2023). The complex and diversified microbiome operates as a functional expansion of host genomes with an estimate of 50-to100-foldmoregenes, these extra genes

contribute to the regulation of host physiology by possessing various types of enzymatic proteins, influencing the produced metabolites and thus affecting host metabolism (Costello et al., 2009)
Over the years, instead of looking into the relationship between one specific microorganism with its host, a holistic approach based on the holobiont theory has been applied (Torday et al., 2020). The beneficial interplay of the host and its microbiome is responsible for maintaining the host's health, whereas disease development is often correlated with microbial disymbiosis, or a shift in the microbiota (Aggarwal et al., 2022). As such, pathogens therefore represent only a tiny fraction of microorganisms, whereby the altered composition of the microbiome promotes the emergence and outbreak of pathogens. The vast majority of microbes are crucial for ecosystem functioning as well as beneficial interactions with other microbes, contributing to population dynamics and functional activities (Jansson & Hofmockel, 2018). Thus, opportunistic pathogens show that host microbe interactions depend not only on the host but also on the entire microbiome (Stevens et al., 2021). The microbiota comprises all living members that form the microbiome, which encompasses bacteria, archaea, fungi, algae, and small protists. The members of microbiome also extend to viruses, phages, and mobile genetic elements one of the most controversial inclusions in the definition of a microbiome (Dekaboruah et al., 2020). However, the microbiome has since been further defined to pertain to not only the community of microorganisms but also the whole spectrum of molecules produced by microorganisms, including their structural elements, metabolites, and molecules produced by the coexisting host (Aggarwal et al., 2022).

Generally, microbial composition varies among different an atomically parts, and it is highly personalized as the microbiome's composition also varies among individuals. The exact definition of a healthy microbiota has yet to be defined, but studies have shown that the use of probiotics, prebiotics, and symbiotic are beneficial by maintaining healthy body flora or by altering the microbiome to ward a healthy microbial ecosystem (Schulze et al., 2021). Accordingly, the coevolution of the microbiome with the host has resulted in these communities playing a profound role in promoting human health. Consequently, perturbations in the human microbiome can cause or exacerbate several diseases (Dekaboruah et al., 2020).

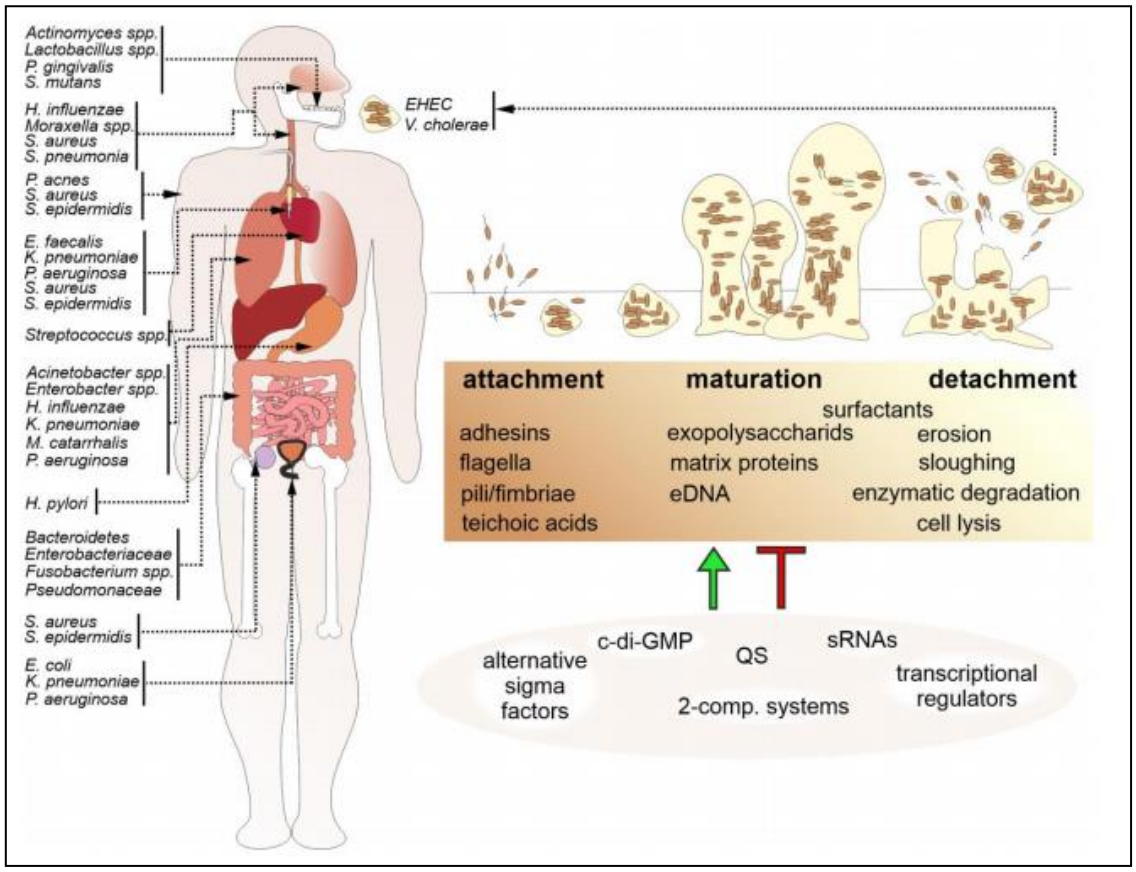


Figure 1: **Distribution of pathogens on various tissues of human body**

A schematic overview indicating representative bacterial species associated with biofilm-related diseases and their occurrence in the body (arrows) is presented above, on the left, Biofilm formation upper right **Figure 1** is a multistep process organized in an attachment, maturation and detachment phase. Biofilm formation is controlled and modulated by several factors including bacterial surface molecules, secreted matrix effectors, as well as environmental components and stressors. Thus, it is not surprising that bacterial biofilm regulation (lower right) involves the interplay of several positive and negative regulatory cascades including quorum sensing systems (QS), regulatory small RNAs (sRNAs), alternative sigma factors, two component systems and second messengers, such as c-di-GMP (Schulze et al., 2021).

I.3. Pathogenic bacteria species with significant biofilm-forming:

Biofilms are three dimensional structures of various bacteria that adhere to biotic or abiotic surfaces. Generally, biofilms are founded by single cells or small groups of cells that then divide and differentiate into complex communities with extracellular matrices water channels embedded extracellular proteins, extracellular lipids and embedded extracellular nucleic acids. Many biofilms also include humic and uronic acids (Gowrishankar et al., 2012) .After adherence to a sur face, bacteria produce a mucilaginous extracellular matrix (ECM), which is absent in planktonic counterparts. ECM sur rounds the biofilm bacteria and contributes to the structure of mature biofilm. Mostly the matrix consists of water (97%), besides it contains exopolysaccharide (EPS) polymer, lipids/ phospholipids, nucleic acids, proteins, absorbed metabolites, and nutrients (Bhowmik et al., 2021).

I.3.1. *Staphylococcus aureus* biofilm :

Biofilms contribute to bacterial fitness by increasing adherence to various surfaces, protection from predation, desiccation, immune attack, antibiotics, and protection from starvation via carbon storage. In addition, it can contribute to pathogenesis and environmental survival of bacteria, biofilms also can have significantly different structural elements (Wu et al., 2024).

Currently, extracellular proteins, carbohydrates and nucleic acids are considered the principal components of biofilm, when these central extracellular components are enzymatically degraded biofilms size can be reduced considerably the substances surrounding the cells in a biofilm are often referred to as extracellular polymeric substances (EPS). While, EPS includes all of the extracellular lipids, carbohydrates ,protein, and acids associated within the biofilm, the majority of current EPS research focuses on the carbohydrate components as they are believed to generally constitute a majority of biofilm biomass (François et al., 2023).

The actual percent biomass contribution is likely dependent upon the nature of the biofilm being studied. The identity of each sugar component, the mechanism of linkage and the order in which the sugars are joined are highly variable across different species and conditions. these carbohydrate chains contribute considerably to the incredible diversity of biofilms found throughout nature (Nguyen et al., 2020) .These complex biofilm structures are associated with disease states, biocorrosion, and biofouling. They are also associated with food production and the maintenance of human health. On a more functional level, biofilms have very different properties than planktonic cells such as increased resistance to antibiotics, antiseptics, disinfectants, protists, phages, shear force, heat, desiccation and UV as well as additional properties (Archer et al., 2011a). While not every studied biofilm has each of the above qualities relative to planktonic form, biofilm has nonetheless been established as a unique state. Recently, multiple transcriptomic studies highlighting the difference between biofilm and planktonic cultures have been performed. (Abdullahi et al., 2016; Kassinger & van Hoek, 2020)

I.3.2. Biofilm extracellular matrix composition and life cycle:

Biofilm formation in *S. aureus* is initiated when free floating, planktonic cells attach to available surface and start colonising. *S. aureus* adherence to a surface is influenced by hydrophobic and hydrophilic interaction between the *S. aureus* cells surface and biotic or abiotic surface. It has been found that the *S .aureus* cell surface adherers to hydrophobic surface by the help of many weakly binding macromolecules, while its adherence to hydrophilic surfaces involves fewer but stronger binding macromolecules (Tuon et al., 2023). The formation of micro colonies is following by the formation of an Extra polymeric substance (EPS) that develops in fully matured biofilm, once the biofilm is fully matured; the bacterial cells residing inside it released certain chemicals i.e., D-amino acids and EPS degrading enzymes such as alginate lyase, to break and disperse the biofilm. These

planktonic cells are ready to either recolonise the same sit or attach to different site and repeat the process of to form a new biofilm (Wu et al., 2024).

Staphylococcus aureus cells that encased and protected by biofilm show different phenotypic characters compared to cells in their planktonic form. Biofilm associated *Staphylococcus aureus* cells are more resistance to antibiotic and exhibit a difference in cell size and growth, genes expression and proteins production, compared to their free-living counterparts (Idrees et al., 2021)

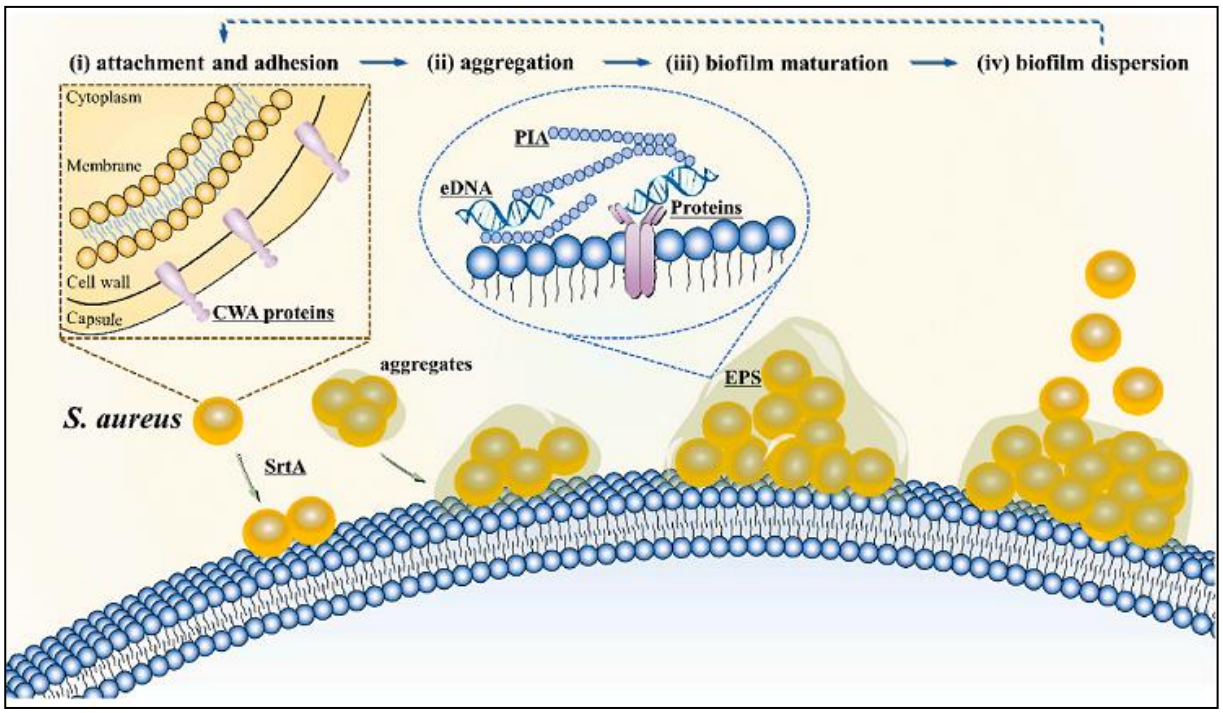


Figure 2: Staphylococcus aureus biofilm formation

(i) initial attachment and adhesion, in which single cells or aggregates adhere to surfaces; (ii) aggregation, with cell division and proliferation as well as EPS production; (iii) biofilm structuring and maturation, where microorganisms coexist within polymicrobial interactions; and (iv) biofilm dispersion, with cell detachment from the aggregate biofilm to planktonic state. EPS, extracellular polymeric substances; eDNA, extracellular DNA; PIA, Polysaccharide intercellular adhesin; CWA proteins, cell wall-anchored proteins; SrtA, sortase A. Key molecules of potential anti-biofilm targets are underlined (Wu et al., 2024)

I.3.3. Biofilm physiology and Quorum sensing:

Numerous Gram-positive and Gram-negative bacteria use Qs signal circuits to coordinate a diverse array of physiological behaviors such as symbiosis, competence, virulence, conjugation, antibiotic production, sporulation, motility, and biofilm formation (Vashistha et al., 2023). The QS system has been divided into two paradigmatic classes: oligopeptide/two component-type quorum sensing circuits in Gram-positive bacteria and Lux I/Lux R-type quorum sensing system in Gram-negative bacteria (Y.-H. Li & Tian, 2012). The difference in regulatory process depends on the chemical structure of signal molecule and its detection mechanism. In general, Gram-positive bacteria use processed oligopeptides and Gram-negative bacteria use AHL as signal molecule to coordinate their behaviors. Furthermore, the molecular bases of the synthesis and perception of different quorum sensing signals and details of the signal transduction pathways have revealed their specific behaviors.

As AHL-mediated quorum sensing system of Gram-negative bacteria is known to be involved in biofilm formations. The most studied system in *S. aureus* is the Agr quorum-sensing system (Shaaban et al., 2019).

Biofilm associated *Staphylococcus aureus* have been reported to have four different metabolic states, i.e., they can either be growing aerobically, can be fermentative, can be dormant, or can even be bead, besides the Extracellular polymeric matrix that shelters the cells against antibacterial agents (Moormeier & Bayles, 2017). *S. aureus* cells encased in a biofilm grow at different rats, i.e., some cells grows at a faster rate as compared to other cells within the same biofilm. This cells are smaller in size and attain their normal size once released upon the dispersal of the biofilm (Archer et al., 2011a). Biofilms seem to be the best strategy for bacteria to survive to any kind of environmental stress, the detection of stress and thus the response needs to be fast enough to survive under those conditions. Therefore, the rapid process of activation of the biofilm program is crucial for the bacteria (Haque et al., 2021).

For *S. aureus*, only one specific QS system was so far described, but most probably, there are other mechanisms for communication. At some point, some genes involved in *S. aureus* virulence were named accessory genes, and an accessory gene regulator was identified as a global regulator of virulence factors genes (T. Li et al., 2016). Different experimental designs have shown that the Agr system induced by an extracellular ligand, the auto-inducing peptides, is a sensor of population and so considered as a QS system. During biofilm formation, Agr QS system is repressed to stop the expression of *S. aureus* colonization factors, and it is activated during the dispersion of the bacteria (Butrico & Cassat, 2020). Moreover, Agr QS system is necessary for the communication inside mature biofilm to establish the three dimensional structure through the control of cell dispersion. This probably requires phenol-soluble modulins, and proteases activated by Agr and involved in the degradation of EPS (Bergey, 1994). However, Agr does not control important biofilm adhesive molecules such as the polysaccharide intercellular adhesions, currently named PIA. One problem underlined each time is the difficulty to detect Agr expression due to the very slow bacteria metabolism in the biofilm (Wu et al., 2024). Other regulators have been identified such as Rbf which is involved in *S. aureus* biofilm formation at the maturation stage rather than at the initial attachment (Butrico & Cassat, 2020).

a) Program on/off

As described for stress response, the setup of inducible processes based on the differential expression of an important number of genes. Biofilm bacteria cells are physiologically different from free cells. Indeed, the different steps as adhesion and immobilization need the expression of various genes (Peng et al., 2022). More important, the communication between bacteria (QS system) controls many metabolic systems and leads to regulation of many genes. The production of the QS molecules as an

endogenous signal leads to changes according to the detected concentration (He et al., 2014). Environmental clues trigger genetic and physiological changes also called biofilm transition. As previously described, the matrix is the plinth of biofilm development and is responsible for many processes in the biofilm program. Moreover, biofilm cells show a general downregulation of their metabolism underlining the slow growing cell or the lack of oxygen due to the biofilm structure, like during fermentation. An upregulation of the urease and the arginine deiminase pathway to limit the side effects of the acidic pH during anaerobic growth was also observed in biofilm structure. All those adaptations participate to a general biofilm setup process (François et al., 2023). The differential gene expressions also lead to antibiotic resistance mechanism. In *S. epidermidis*, some of these antibiotic resistance mechanisms are upregulated during biofilm stage. In *S. aureus*, Agr (accessory gene regulator) expression and involvement in biofilm formation depend of the environmental conditions. The agr expression shut down has no effect, enhances or inhibits biofilm formation according to the environmental parameters. Biofilm program is a temporary response to stress conditions and this process is able to turn off quite quickly when conditions are more favourable for the bacteria (Archer et al., 2011a; François et al., 2023).

b) Interactions with the environment and survival strategy

Bacteria have the extraordinary ability to survive in any harsh conditions, and as recently discovered, this is due to their capacity to form biofilm. Many environments can be a source of stress for bacteria (Vashistha et al., 2023). *S. aureus* biofilm have been found in industry and in clinical domain, particularly in biofilm-associated infections. Environmental stresses are supposed to induce biofilm formation. As evidence, sigma B, a protein required for transcription and activated under stress responses due to heat shock, MnCl2, NaCl2 and alkaline shock, is involved in biofilm formation (Moormeier & Bayles, 2017).

In *S. aureus*, nutrients like glucose or NaCl can influence biofilm. For example, Rbf regulator is involved in biofilm formation under high concentrations of glucose and NaCl conditions, but not in the presence of ethanol (Cue et al., 2009). Nutrient starvation has been underlined as an important environmental stress, which could induce biofilm maturation. In vitro, however, the addition of glucose is required for biofilm formation and activation of the agr QS system, even if oldest results showed the contrary. In fact, conditions to form biofilm seem to be very specific, such as a balance between an over concentration of glucose and a lack of carbon source. The pH maintenance also influences Agr system and, in consequence, probably acts on biofilm formation (Butrico & Cassat, 2020).

c) Interactions with the host immune cells

During bacterial infection, host immune cells are the defenders of the organism. Through mechanisms such as phagocytosis or release of bactericidal components, these cells are able to fight and neutralize

planktonic S. aureus. Concerning *S. aureus* biofilm, the general thought is that biofilm structure protects the bacteria against the immune cells, avoiding interaction between both actors (Peng et al., 2022). Nevertheless, recent studies reported that polymorphonuclear neutrophils (PMN), macrophages, myeloid derived suppressor cells (MDSCs) and T lymphocytes can interact with S. aureus biofilm in a double-edged interplay (Nguyen et al., 2020). PMNs are the first line of defence in bacterial infections. These cells can phagocyte planktonic bacteria and release bactericidal components such as reactive oxygen species or enzymes. Contrary to the dogma (Reffuveille et al., 2017). In the context of *S. aureus* biofilm infection, in vitro and in vivo studies reported that invasion of macrophages into biofilms is limited. *S. aureus* biofilms is able to secrete specific toxins. Interplay between *S. aureus* biofilm and host immune cells called alpha-toxin (Hla) and leukocidin AB (LukAB) that inhibit macrophage phagocytosis and induce cytotoxicity, promoting macrophage dysfunction and thus facilitating *S. aureus* biofilm development. (Butrico & Cassat, 2020).

I.3.4. Genetic regulation of biofilm:

S. aureus can produce a multilayered biofilm embedded within a glycocalyx or slime layer with heterogeneous protein expression throughout, the solid component of the glycocalyx as primarily composed of teichoic acids (80%) and staphylococcal and host proteins. The specific polysaccharide antigen PIA composed of b-1,6-linked N-acetylglucosamine residues (80–85%) and an anionic fraction with a lower content of non-N-acetylated D-glucosaminyl residues that content of non-N-acetylated D-glucosaminyl residues that contains phosphate and ester-linked succinate (15–20%) (Archer et al., 2011a)

a) PIA-dependent biofilm formation:

PIA is produced in vitro from UDP-N-acetylglucosamine via products of the intercellular adhesion (ica) locus. The genes and products of the ica locus [icaR (regulatory) and ica ADBC (biosynthetic) genes] have been demonstrated to be necessary for biofilm formation and virulence and are upregulated in response to anaerobic growth, such as the conditions seen in the biofilm environment (Jefferson et al., 2004). The staphylococcal respiratory response regulator, SrrAB, is responsible for PIA induction under anaerobic environments via binding of a 100 bp DNA sequence upstream of the icaADBC operon. Other environmental factors can also play a role in regulation of ica, including glucose, ethanol, osmolarity, temperature and antibiotics such as tetracycline (Avila-Novoa et al., 2018). In the homologous *S. epidermidis* locus, regulation of Ica can occur via reversible inactivation by insertion sequence (IS256) phase variation in 25–33% of variants, and this has been observed in some S. aureus strains as well. In addition, PIA expression is repressed by IcaR, a transcriptional regulator of *the* teicoplanin associated locus; however ,deletion of the IcaR gene had no effect on PIA synthesis (François et al., 2023). IcaR confers strong negative regulation, through binding of the Ica cluster promoter and deletion of the IcaR gene results in enhanced Ica cluster gene expression. The

protein regulator of biofilm formation, Rbf, however, represses transcription of IcaR, albeit indirectly, leading to augmented Ica gene expression, PIA production and biofilm formation (Peng et al., 2022). In addition, Spx, a global regulator of stress response genes, was shown to have a negative regulatory impact on biofilm formation, seemingly by modulating IcaR (Archer et al., 2011b; Boles et al., 2010; Cue et al., 2009; Jefferson et al., 2004).

a) PIA-independent biofilm formation:

Despite the importance of the ica gene locus in biofilm development, biofilms can occur in an ica-independent fashion. The arlRS two component system was shown to repress biofilm development, and when deleted led to enhanced attachment and PIA production. However, biofilm synthesis was unaffected by additional deletion of the IcaADBC operon, suggesting that in this double deletion mutant, PIA was not essential for biofilm development (Nguyen et al., 2020). The *S. aureus* clinical isolate, UAMS-1 (University of Arkansas Medical System-1), had unabated biofilm formation in vitro and in vivo in a catheter infection model even with mutation of the ica cluster. In a guinea pig model of biofilm infection, deletion of Ica and thus, lack of PIA production caused no decrease in virulence. In addition, Fitzpatrick et al. showed that biofilm formation in MRSA strain BH1CC was unaffected by Ica locus deletion (Boles et al., 2010). However, other mutant strains lost the ability to form biofilm. Interestingly, when S. aureus icaADBC operon deletion mutants are categorized by methicillin susceptibility, MRSA strains are capable of biofilm development, whereas MSSA strains are impaired in biofilm formation (Archer et al., 2011a). These data propose that biofilm formation in an Ica-independent manner is strain specific. In an ica-deletion mutant *S. aureus* strain, protein A (SpA) production was found essential for biofilm formation.34 Furthermore, biofilm development could be recovered in spa mutants by addition of exogenous SpA, indicating that it is not necessary for SpA to be covalently anchored to the cell wall. The fibronectin-binding proteins (FnBPs) can also arbitrate biofilm formation through an essential role by the major autolysin (Atl) and sigB regulation, and in *S. epidermidis*, PIA-independent biofilms were mediated through the accumulation-associated protein (Aap) (Peng et al., 2022). In addition, biofilm associated protein (Bap) and Bap-related proteins of *S. aureus* can confer biofilm development independent of PIA production through cell to cell aggregation, and are characterized by their high molecular weight, presence on the bacterial surface, role as a virulence factor and occasional containment in mobile elements (Nguyen et al., 2020). These reports suggest that proteinaceous cell-to-cell adhesion can substitute PIA mediated biofilm development in ica independent biofilms. (Avila-Novoa et al., 2018; Lister & Horswill, 2014)

I.4. *Pseudomonas aeruginosa* biofilm:

Among the majority of available gram negative bacteria, *Pseudomonas aeruginosa* is one of the most noticeable bacteria known to cause harmful infections via biofilm formation (J. Yadav et al., 2021). which promotes their survival in the environment, a variety of hostile conditions This is the most

common cause of worldwide microbial, chronic, and nosocomial infections unlike other common gram negative bacteria, *P. aeruginosa* has a remarkable ability to infect a large number of humans (Karami et al., 2020).

I.4.1. Biofilm composition:

The biofilm matrix components that have been identified from *P. aeruginosa* mainly include exopolysaccharides, eDNA, and matrix proteins, which play an important role in the structural maintenance and drug resistance of biofilms. *P. aeruginosa* can synthesize at least three types of exopolysaccharides: alginate, Pel polysaccharide, and Psl polysaccharide. Alginate is an anionic polysaccharide of α-L-guluronic acid and β-D-mannuronic acid linked by β-1-4 glycosidic bonds (Colvin et al., 2012). The overproduction of alginate is responsible for the development of excessive slimy or mucoid biofilms, while mucoid biofilms are more resistant to host immune system attack and antibiotic treatment than non-mucoid biofilms (Jennings et al., 2015) The role of Pel and Psl in biofilm formation can vary drastically. For example, the two most commonly studied non-mucoid laboratory strains, PAO1 and PA14, differ in the primary polysaccharide used to maintain biofilm structure. PAO1 relies primarily on Psl, while Pel production is required for mature biofilm development in PA14. Collectively, these studies suggest that Pel and Psl are each capable of functioning as a structural scaffold involved in maintaining biofilm integrity (Grossich et al., 2023).

I.4.2. Biofilm life cycle:

P. aeruginosa has been demonstrated to grow slowly as unattached cell aggregates under hypoxic and anoxic conditions, slow growth rates in the limited presence of oxygen are ascribed to antibiotic recalcitrance. The biofilm development is divided into five distinct stages (Figure3) .Stage I: Bacterial cells adhere to a surface via support of cell appendages such as flagella and type IV pili. The restricted flagellar movement has been implicated in mediating twitching motility and biosynthesis of exopolysaccharides required for surface association which is reversible adherence (Thi et al., 2020) (**fig. 03**).

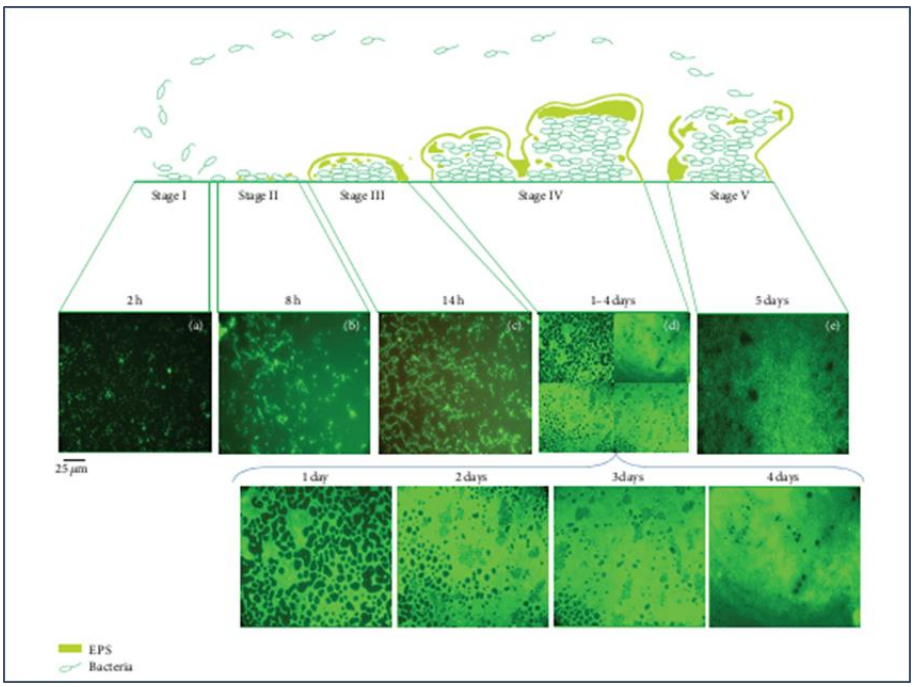


Figure 3: Biofilm lifestyle cycle of Pseudomonas aeruginosa

P. aeruginosa PAO1 grown in glucose minimal media. In stage I, planktonic bacteria initiate attachment to an abiotic surface, which becomes irreversible in stage II. Stage III corresponds to microcolony formation. Stage IV corresponds to biofilm maturation and growth of the three-dimensional community. Dispersion occurs in stage V and planktonic bacteria that are released from the biofilm to colonize other sites. The biofilm formation by P. aeruginosa PAO1 was revealed with Syto9 and visualized in Leica DM IRE2 inverted fluorescence microscope with 400x magnification at 2h (Stage I), 8 h (Stage II), 14h (Stage III), 1 to 4 days (Stage IV), and 5 days (Stage V). Images represent a 250 ×250-μm field (Rasamiravaka et al., 2015)

I.4.3. Biofilm physiology and Quorum sensing:

Pseudomonas aeruginosa biofilm is one of the most studied biofilm models. A powerful system of communication between cells was described in P. aeruginosa biofilm and named “quorum-sensing” .First the quorum-sensing system was linked to a communication based on cell density. Then, quorum sensing is virtually connected to biofilm formation and dispersal phenomena. The communication system in *P. aeruginosa* is based on molecules called acylhomoserine lactones (AHLs) which penetrate bacteria and directly regulate target gene. In *P. aeruginosa* QS may also increase the resistance to oxidative stress stimuli by increasing the expression of catalase and superoxide dismutase.

I.4.4. Genetic regulation of biofilm:

P. aeruginosa produces at least three extracellular polysaccharides that can be important in biofilm development. Alginate is an important biofilm exopolysaccharide that is over produced in mucoid variants. In mucoid strains, alginate is the predominant extracellular polysaccharide of the matrix. Non-mucoid strains utilize primarily the Pel and Psl polysaccharides for biofilm formation (Grossich et al., 2023). The pel locus contains seven genes encoding functions involved in the synthesis and export of an uncharacterized polysaccharide. The pel locus was identified in a transposon mutagenesis screen for loss of pellicle formation, a biofilm formed at the air-liquid interface of a

static liquid culture. The loss of biofilm formation is specifically attributed to the capability of Pel to initiate and maintain cell-cell interactions (Colvin et al., 2012).

The Psl polysaccharide consists of 15 co-transcribed genes (pslA to pslO) that encode proteins to synthesize Psl, enhance cell-surface and cell-to-cell adhesion in P. aeruginosa, and play an important role in the initiation and maintenance of biofilm structure. Pel is a positively charged exopolysaccharide composed of partially acetylated 1→4 glycosidic linkages of N-acetylgalactosamine and N-acetylglucosamine, which is important for biofilm formation in air–liquid interfaces Pel and Psl are the major structural polysaccharides in non-mucoid and mucoid *P. aeruginosa* biofilms (Grossich et al., 2023). Cell lysis releases DNA into the environment and this eDNA can be used as a supporting component of biofilms to provide nutrients to bacteria in biofilms during periods of nutrient deficiency. Aside from exopolysaccharides and eDNA, extracellular proteins are also considered to be important components of biofilm matrices, including appendages (mainly flagella and type IV fimbriae), cytoadhesions, and lectins. Studies have found that these components mainly play an auxiliary role as adhesion factors and structural support in the process of *P. aeruginosa* biofilm formation (Yin et al., 2022).

Regulation of Pel and Psl expression is complex, with multiple levels of intricate control. Recent studies have demonstrated multiple pathways of transcriptional control for both *pel* and *psl*. FleQ represses transcription of the *pel* and *psl* operons (Colvin et al., 2012). This repression is relieved in the presence of the intracellular signaling molecule c-di-GMP. RpoS acts as a positive transcriptional regulator of *psl* gene expression and quorum sensing has been suggested to positively regulate *pel* and *psl* expression as well (Irie et al., 2010). Another regulatory system controlling *pel* and *psl* gene expression is the *Gac-Rsm* signal transduction pathway. The RNA binding protein and *RsmA* inhibit translation of *psl*. Finally, c-di-GMP can act as a positive allosteric regulator of Pel synthesis through PelD binding (Grossich et al., 2023).

I.5. *Escherichia coli* biofilm :

Escherichia coli is a common bacterial species and in close relation with humans and many animals as a normal fora of gastrointestinal tract (Perry & Tan, 2023). However, some strains acquire specific virulence factors (VF), which make a capacity of causing infection disease (Clark et al., 2019). As the intestinal and extra-intestinal infections, including diarrhea and urinary tract infections (UTI). In addition, extra-intestinal pathogenic *E. coli* (ExPEC) which holds VFs are enable to colonize in the urinary tract mucosa and invade following overcome the host immune defences (Ebrahimi et al., 2023)

I.5.1. Biofilm composition and life cycle:

E. coli is a well-characterized bacterium that plays an essential role in the human microbiome. However, some strains can become pathogenic and cause infections not only in the intestinal tract but also in other parts of the human body where they could form a biofilm (Schulze et al., 2021). Biofilm formation in *E. coli* is a complex developmental process that occurs in different phases: reversible and irreversible attachment, maturation, and dispersion.

a) Reversible Attachment:

In the first phase of biofilm formation, *E. coli* must move in liquid or semi-solid media to find suitable surfaces with favorable conditions for attachment. For this purpose, *E. coli* uses flagella that allow the bacteria to swim and approach the surface by rotating clockwise and counterclockwise. In addition, the flagella enable the cell to overcome the effects of repulsive forces (such as hydrodynamic and van der Waals forces) between the bacteria and the surface, allowing them to arrive and attach to the surface (Ballén et al., 2022).

b) Irreversible Attachment:

Once *E. coli* is reversibly attached, adhesion to the surface can become irreversible if the environmental conditions are suitable for a sessile lifestyle. This transition from reversible to irreversible attachment is a regulated process that gives *E. coli* the ability to analyze the local environment before transitioning to a biofilm state. To carry out this irreversible attachment, *E. coli* uses three types of organelles: conjugative pili, curli fibers, and type 1 fimbriae.

c) Maturation:

During biofilm maturation, matrix production begins, allowing the development of structured communities and determining the final architecture and spatial arrangement of the biofilm. The matrix provides biofilm stability, promotes intercellular interaction, and enables the transport of nutrients and waste through the biofilms. In addition, the biofilm matrix serves as a protective barrier against the adverse effects of desiccation, antimicrobial agents, antibodies, and host immune response, including complement action and phagocytosis (Öztürk et al., 2023).

d) Dispersion:

The dispersion step is the final phase of biofilm development. This phase promotes the detachment of the bacteria from the biofilm and allows their dispersal in the environment and subsequent colonization of new surfaces or niches. Environmental conditions, such as low nutrient and oxygen availability, pH changes, high concentrations of toxic products ,and other stress conditions can promote biofilm spread (Zhou et al., 2022). The release of cells from biofilm is mediated by two mechanisms:

- Dispersion is an active process in which bacteria escape from the biofilm through enzymatic degradation, leaving eroded biofilms behind and allowing bacteria to spread to new sites (Ballén et al., 2022).

- In the case of the passive detachment, external factors such as fluid shear forces ,abrasion, and human disturbance act as triggers for this process (Ballén et al., 2022)

I.5.2. Biofilm physiology and Quorum sensing:

Quorum sensing (QS) is an intercellular signalling mechanism that allows the communication between bacteria in a cell density-dependent manner. The QS signalling system enables the bacteria to modify their gene expression pattern in response to changes in the environmental conditions, such as nutrient starvation, alterations in temperature, pH and osmolarity, oxidative stress, membrane stresses, antibiotics, and other toxic substances (Escobar-Muciño et al., 2022). It provides the bacteria with a selective survival advantage under different harsh conditions .Among others, the QS signalling cascade modulates cellular functions such as metabolic activity; extracellular polymeric substance (EPS) production, nutrient acquisition, transfer of genetic material between the cells, motility, biofilm formation, antibiotic resistance, virulence, and the synthesis of secondary metabolites (Kamath et al., 2023). The QS system usually involves the secretion of small molecules (auto-inducers) that act on surface receptors on adjacent bacteria resulting in the induction of signal transduction pathways regulating biofilm formation, virulence, competence, conjugation, antibiotic resistance, motility, and sporulation (Sionov & Steinberg, 2022).

I.5.3. Genetic regulation of biofilm:

In *E. coli* and related bacteria this matrix consists of proteinaceous components including various adhesins as well as amyloids such as curly fibers, which can be interwoven with the exopolysaccharides cellulose, poly-β-1,6-D-N-acetylglucosamine (PGA) and colanic acid. The composition of the biofilm matrix varies depending on temperature, growth conditions and genetic background of the strains (Yan et al., 2023). In pathogenic *E. coli* living within the host or on abiotic surfaces (37 °C), type I fimbriae or the adhesin AG43 are involved in initial attachment, PGA stabilizes permanent attachment and also curly fibers, which contribute to surface attachment, can be a predominant matrix component (Öztürk et al., 2023). Bacteria growing in the environment or on abiotic surfaces at lower temperatures (<30 °C), form differently composed biofilms, using flagella for initial attachment and curli fibers, cellulose and colanic acid as a matrix in the mature biofilm (Mika & Hengge, 2013).

In *E. coli*. biofilms grown for 24 h, DNA housekeeping genes dam and maoP were seen to have a significant effect on biofilm fitness, the author has identified a novel role for maoP in biofilm formation, demonstrating its deletion resulted in a reduction in curli production and biofilm biomass in *E. coli* (Öztürk et al., 2023). The role of this gene on biofilm fitness in another member of the Enterobacteriaceae family, Antitoxin modulator tomB was found to benefit the fitness of *E. coli* biofilms grown for 12, 24 and 48 h (Holden et al., 2022).

I.6. Biofilm medical impact and biofilm on medical dispositive:

Biofilms have both positive and negative impacts on public health issues, they can be beneficial by protecting our bodies from certain harmful agents present in an environment through remediation of soil and groundwater (Reffuveille et al., 2017). However, biofilms are considered detrimental agents to our health. In medical device, biofilms aid microbes to easily adhere to indwelling medical devices (IMDs) such as contact lenses, central venous catheters, mechanical heart valves, peritoneal dialysis catheters, prosthetic joints, pacemakers, urinary catheters, voice prostheses, intravascular catheters, dental inserts, breast implants, and orthopedic inserts are a potential risk of t pathogens forming biofilm for patients following these devices (Assefa & Amare, 2022). Biofilms can also grow on biotic surfaces, such as teeth, lungs and bone. Biofilms that grow in water systems supplying healthcare facilities are a serious problem, such as biofilms of *P. aeruginosa* in metal water pipes Such biofilms transferred to an individual are usually related to life-threatening infections, such as cystic fibrosis, periodontitis, infective endocarditis, otitis media, osteomyelitis and chronic wounds (Karami et al., 2020).

Biofilms account for up to 80% of microbial infections according to the National Institutes of Health (NIH). Staphylococcal species are the leading cause of implantable device-associated infections Biofilms play a significant role in various diseases (François et al., 2023; Idrees et al., 2021) , such as chronic respiratory infection, chronic lung disease, chronic obstructive pulmonary disease and ventilator associated pneumonia. Secondary infections can sometimes cause severe bacteremia or septicemia after biofilm organisms enter into the blood through implanted devices (Mirghani et al., 2022).

Pseudomonas aeruginosa is an opportunistic human that causes nosocomial infections such as pneumonia, bacteremia, and infections of the lesions, corneas, gastrointestinal tract, and urinary tract (Yin et al., 2022). Furthermore, it deteriorates the health of immunocompromised people, Cystic Fibrosis patients, Human Immunodeficiency Virus carriers, and cancer patients (Martegani et al., 2020),. *P aeruginosa* can form biofilm on pyrolytic carbon heart valves, pacemakers, contact lenses, urinary catheters, and central venous catheters. This can lead to serious chronic infections such as endophthalmitis, malignant external otitis, endocarditis, meningitis, and septicemia, putting patients' lives at risk (J. Yadav et al., 2021). *P. aeruginosa* pathogenicity is realized by a large number of secreted virulence genes and factors, such as toxins and enzymes. The type III secretion system secretes several genes involved in the production of exotoxins (exoS, exoT, exoU, and exoY). These cytotoxins play a role in bacterial evasion of host immune responses, inhibition of DNA synthesis, and, as a result, host cell death (Ghazalibina et al., 2019)

I.6.1. Antibiotic resistance and biofilm related multidrug resistance (MDR)

According to the 2022 World Health Organization (WHO) report, antimicrobial resistance (AMR) is prevalent and can influence individuals of any age, in any country of the world (Dong et al., 2023). The consequences of unchecked AMR are wide ranging and extremely costly, not only financially, but also in terms of global health, food security, environmental well-being, and socioeconomic development (Chang et al., 2015). Enzymatic hydrolysis, enzymatic modification of antibiotics by group transfer and redox process, modification of antibiotic targets, reduced permeability to antibiotics by modification of porins and active extrusion of antibiotics by membrane efflux pumps are the most common cellular mechanisms underlying antibiotic resistance (Tuon et al., 2023).

The increased use of antibiotic treatments has led to the spread of antibiotic resistance genes by horizontal gene transfer or the selection of vertically transmitted mutations, Whereas the horizontal transfer of antibiotic resistance genes is well established in biofilms (Coenye et al., 2022).

The microbial biofilm is the main mechanism of drug resistance that contributes to the emergence of MDR microorganisms. Because of the restricted penetration of antibiotics into the biofilm matrix, high cellular density, quorum sensing abilities, the decreased growth rate of bacteria in the biofilm, an elevated expression of efflux pumps, high mutation frequency to develop new strain, the presence of persistent cells, and overexpression and exchange of resistance genes among bacteria within a biofilm (Assefa & Amare, 2022).

Biofilms indeed display a characteristic high level of tolerance to a broad range of antibiotics that disappears quickly after biofilm dispersion. Consequently, even when caused by non-resistant bacteria, biofilm-associated infections are difficult to eradicate and regrowth of surviving biofilm bacteria when antibiotic treatment stops is a typical cause of therapeutic failure due to bacterial infection relapse. The emergence of antibiotic resistance within a tolerant biofilm population could therefore constitute an aggravating factor increasing the frequency of therapeutic failure and infection recurrence (Assefa & Amare, 2022; Dong et al., 2023).

Methicillin-resistant S. aureus (MRSA) has developed from methicillin-susceptible S. aureus (MSSA) by acquisition of the gene mecA. This gene mediates the production of a beta-lactamase enzyme that inactivates both beta-lactamase-stable drugs (e.g. methicillin and cloxacillin) and beta-lactamase inhibitors (e.g. sulbactam) (Ciandrini et al., 2020). Since its discovery, MRSA strains widely spread through all regions of the world. In 2014, the WHO reported that 86% of the clinical isolates of S. aureus were resistant to methicillin (MRSA) (Mirghani et al., 2022). Patients infected with MRSA have an increased mortality rate and require more healthcare resources than MSSA-infected patients representing a high health and economic burden require. (Idrees et al., 2021).

Uropathogenic *Escherichia coli* is the most common cause of urinary tract infections, accounting for approximately 80% of infections .The routine therapy of urinary tract infections is based on the use

of antibiotics such as β-lactams, trimethoprim, nitrofurantoin and quinolones in many countries. Overuse and misuse of these antibiotics increase the development of resistant bacteria. Particularly, the emergence of uropathogenic multidrug-resistant (MDR) E.coli strains that produce extended spectrum β-lactamases (ESBL) is a serious global health problem, since it can cause prolonged hospital stay, increasing morbidity, mortality, and health care costs (Bush & Bradford, 2020). ESBLs are a group of β-lactamase enzymes that confer resistance to third generation cephalosporins, such as ceftazidime and ceftriaxone. Resistance genes coding for β-lactamases are often located on plasmids which also harbor resistance genes for non- β-lactam antibiotics such as aminoglycosides and trimethoprim-sulfamethoxazole (Sahle et al., 2022).. Therefore, ESBL producing bacteria are commonly MDR, leaving limited antibacterial options (Vazquez et al., 2020)

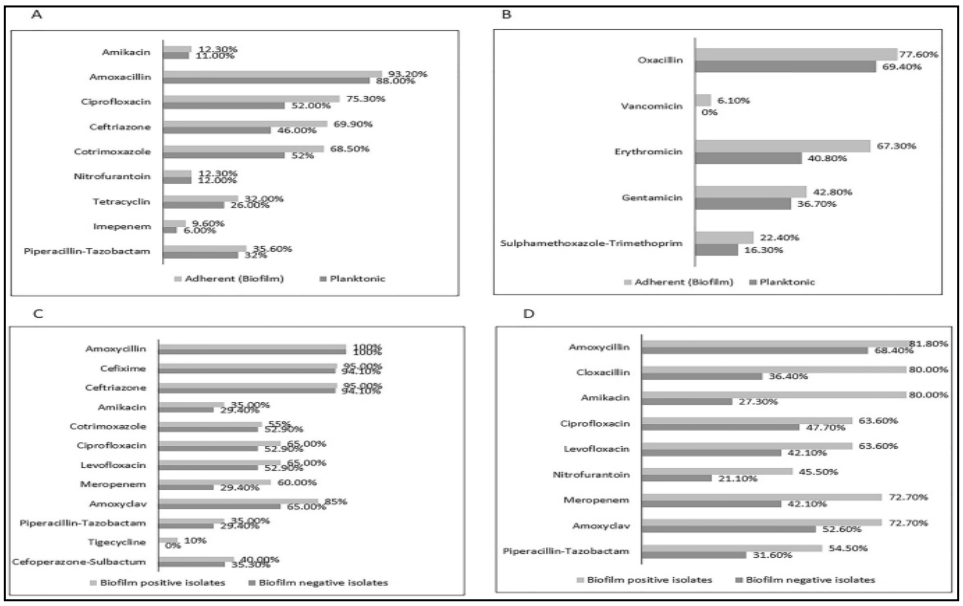


Figure 4: Antibiotic resistant profiles in planktonic and biofilm producing bacteria

Figure 4: Antibiotic resistant profiles in planktonic and biofilm producing

(a) *E.coli*, (b) *Staphylococcus spp.* (c) Gram -negative isolates, and (d) Gram-positive isolates respectively (Koley & Mukherjee, 2021).

I.7. Heterogeneous populations in mixed biofilms:

In vitro, most biofilm studies are examined single biofilm species cultures, whereas in nature, almost all biofilm communities comprise a variety of microorganisms, the species that constitute a mixed biofilm and the interactions between these microorganisms critically influence the development and the structure shape of the sessile microbial community (S. Elias & Banin, 2012).

The mixed biofilm harbors different micro-niches that display a wide genetic and physiological heterogeneity among the bacterial population, the diversity in biofilm is maintained by two distinct inherent properties of the populated cells as well as the ecological competition among them (Shree et al., 2023).

The gene expression profile in biofilm is highly dynamic in comparison with the planktonic lifestyle. To elucidate the community behavior of bacteria in biofilm, understanding their diverse heterogeneity along with the mechanisms involved in competitive interactions within multispecies communities is crucial (Bhowmik et al., 2021). As a result, staphylococcal biofilms are characterized by containing cells with different metabolic profiles: cells growing with active respiration, cells growing fermentatively, dead cells and cells growing with a reduced metabolic activity (dormant cells).

Indeed, this heterogeneity promotes the emergence of cell subpopulations with physiological characteristics, which render them resilient to certain antibiotics. Related to this aspect, it is important to note that many antibiotics target only actively growing cells and require an active metabolism of the target cells to be functional. Therefore, dormant cells, characterized by a low metabolic activity and a slow growth phenotype, are generally resistant to many different antibiotics (Huemer et al., 2020).

Tolerant and dormant cells, the latter also referred to as persister cells, can withstand high antibiotic concentrations, and are commonly found in staphylococcal biofilms. Tolerant cells are able to survive at high antibiotic concentrations during a transitory period without affecting the minimal inhibitory concentration, this ability is a consequence of a mutation or environmental conditions (Dewachter et al., 2019). In contrast, persisters are a small bacterial subpopulation with the ability to survive adverse conditions. This characteristic is therefore not linked to genetic mutations, but instead to a temporary phenotypic variation (Kranjec et al., 2021).

Numerous ecological interactions among microbes for example, competition for space and resources, or interaction among phages and their bacterial hosts are likely to occur simultaneously in multispecies biofilm communities (Jo et al., 2022; Wimpenny et al., 2000). While biofilms formed by just a single species occur, multispecies biofilms are thought to be more typical of microbial communities in the natural environment. Previous work has shown that multispecies biofilms can increase, decrease, or have no measurable impact on phage exposure of a host bacterium living alongside another species that the phages cannot target (Winans et al., 2022).

I.8. Growing biofilms in the laboratory and biofilm detection methods

While bacteria have been studied in the laboratory for well over 100 years, biofilms were first studied after surface-attached bacteria were observed attached to the pacemaker lead in a patient suffering from recurrent bacteremia and growing on glass slides inoculated with seawater. The bacteria attached to the pacemaker lead mark one of the first references to “biofilm growing bacteria” in medicine, with a subsequent explosion of interest in biofilm infections (Merritt et al., 2011). Such studies led to a key publication in the field describing the developmental stages of *P. aeruginosa* (a nosocomial pathogen), presenting the current influential “5-step biofilm model”. While the schematic

conceptual biofilm developmental model based on *P. aeruginosa* in vitro biofilm formation is easy to understand and has been widely generalized to describe all biofilms, this model does not necessarily describe the complexity of biofilms in real world industrial, natural and clinical settings. Importantly, this model does not reflect the relevant microenvironments that develop within these biofilms (Thi et al., 2020).

In such diverse systems, the processes of attachment, aggregation, interaction with biotic or abiotic materials and interfaces (e.g., roots, tissue, a gas phase, environmental polymers, corrosion deposits), growth and maturation, and detachment/dispersal are potentially quite different and do not necessarily occur sequentially. Given the variety of systems and conditions, we propose it would be useful to expand the existing model to include a wider spectrum of real-world scenarios (Sauer et al., 2022).

I.9. Biofilm detection methods

I.9.1. Microtiter Plate Assay (MTP):

Microplate biofilm formation is arguably the most widely used method in the world. Simple to implement and inexpensive, requiring no specific equipment, it allows the passage of a large number of strains and is suitable for screening anti-biofilm molecules. Originally described by Christensen et al in 1985, its principle is based on the formation of biofilm in the 96 wells that make up the plate (Christensen et al., 1985). After the necessary incubation time in an adequate culture medium, the microplate is rinsed using a pipette or by immersion, in order to eliminate the planktonic bacteria. Bacteria form their biofilm at the bottom of the wells, making the use of microplates perfectly adapted since they provide a large surface on which the bacteria will adhere. Conversely, some bacteria form their biofilms at the air-liquid surface. This is the case, for example, of P. aeruginosa, or Bacillus cereus .There Quantification of the biofilm is then done on the ring formed on the edge of the wells (Amran et al., 2024; Berger et al., 2018).

Generally, sessile bacteria adhered to the wells are highlighted by staining .Several dyes exist, the most common being Crystal Violet, Safranin Red, and Congo Red. After a variable contact time depending on the dye (generally a few minutes), the surplus is eliminated by rinsing. After resuspension, it is possible to quantify the biofilm formed by a simple absorbance reading (Coffey & Anderson, 2014). proposed a fixation step addition to ethanol, increasing the reproducibility of the method, as well as a classification of strains based on control values Commonly used dyes are cationic and bind to all the negative charges of the constituents, coming both from the bacteria and the matrix, and therefore do not allow differentiation between the two. An alternative to staining consists of enumerating after rinsing the bacteria still adhered to the bottom wells (Amran et al., 2024).

I.9.2. Tube adherent method (TAM):

Tube adherent method (TAM) that is a qualitative assay for detection of biofilm producer microorganism, as a result of the occurrence of visible film, is described by Christensen et al. Isolates are inoculated in polystyrene test tube which contained TSB and incubated at 24 h at 37°C. The sessile isolates of which biofilms formed on the walls of polystyrene test tube are stained with safranine for 1 h, after planktonic cells are discharged by rinsing twice with phosphate-buffered saline (PBS). Then, safranine-stained polystyrene test tube is rinsed twice with PBS to discharge stain. After air drying of test tube process, the occurrence of visible film lined the walls, and the bottom of the tube indicates biofilm production (Christensen et al., 1982)

I.9.3. Congo Red Agar method (RCA):

Congo red is a diazo textile dye that has been used for nearly a century to visualize the development of amyloid fibers. Later, microbiological uses emerged, particularly in detecting bacteria that form amyloid appendages known as curli and overexpressing polysaccharides in the biofilm matrix. The second is because the messenger cyclic diguanylate (c-di-GMP) governs the formation of biofilm matrix polysaccharides, Congo red staining of samples can be used to evaluate enhanced c-di-GMP production in bacteria. Congo red enables the identification of strains that produce high levels of c-di-GMP in a low cost, quantitative, and high-throughput method (C. J. Jones & Wozniak, 2017).

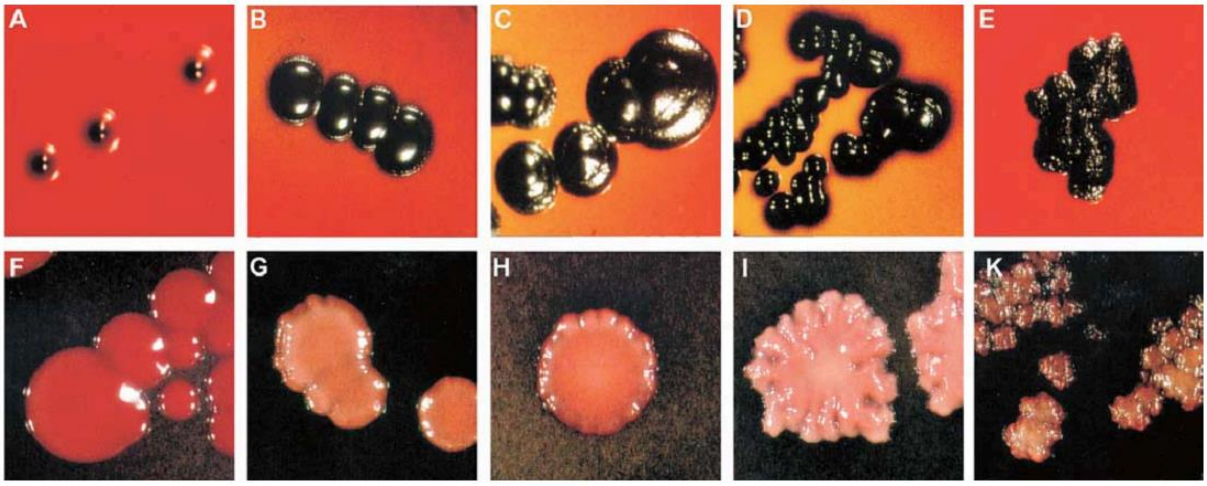


Figure 5: Colony morphology of S. aureus on CRA

Figure 5: Colony morphologies of S. aureus on CRA. Different S. aureus isolates were cultivated on CRATSB (A–E) or CRA BHI (F –K). Four biofilm-positive strains had the typical dry crystalline morphology seen in E and K. All other strains had morphologies consistent with biofilm-negative S. epidermidis strains (A, F)or intermediate morphologies (B –D , G – I) not correlating with their biofilm phenotype (CRA Congo red agar, TSB trypticase soy broth, BHI brain heart infusion) (Knobloch et al., 2002).

I.9.4. The Biofilm Ring Test (BFRT):

Is a rapid and simple method to assess the ability of microorganisms to form biofilm. It is based on the direct measurement of the mobility of magnetized microbeads become trapped in the biofilm and lose their mobility, resulting in the absence of a spot of microbeads in the well bottom of the test. The BFRT is performed by dispersing a suspension of magnetized in a well of a microtiter plate. Then the well is inoculated with the microorganism of interest for a period of time. typically 24 to 48 h. after incubation, the microbeads are attracted to a magnet placed at the bottom of the well, and the presenceor absence of a spot of microbeads is observed, if a spot of microbeads is present, it indicates that the microorganism have no ability to form biofilm. If there is no spot of microbeads, it indicates that the microorganism formed a biofilm (Chavant et al., 2007). The BFRT has been showed to be a reliable and reproducible method for assessing biofilm formation by a variety of microorganisms. It is particularly useful for assessing the biofilm formation potential of microorganisms in clinical and environmental samples. The BFRT is a valuable tool for researchers and clinicians who need to quickly, and easily assess the biofilm formation potential of microorganisms. It can be used to evaluate the efficacy of biofilm control strategies, to identify microorganism with high biofilm formation and monitor the biofilm formation status of patients with chronic infection (Chavant et al., 2007; Olivares et al., 2016, 2020).

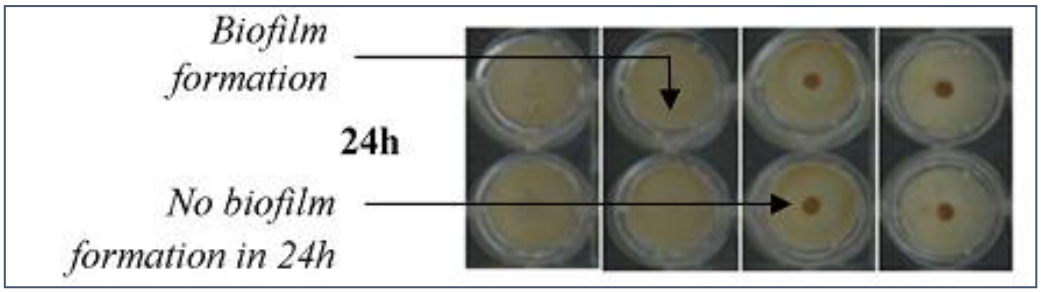


Figure 6: Kinetic of biofilm formation with the BioFilm Ring Test

three selected *P. aeruginosa* strains with the BioFilm Ring Test. Controls with BHI medium and toner alone. Well images were obtained by scan of microplates with the plate reader after magnetization by the block test (Olivares et al., 2016).

I.9.5. Genetic Biofilm Screening Model

These methods are very useful for quantification of biofilm from environmental samples and static or flow systems and allow study polimicrobial biofilms attached to different surfaces.

A. Real time quantitative-reverse transcription- PCR (qRT-PCR)

qRT-PCR has been proposed as a promising indicator of cell viability because can detects all cells in a sample, including the dead cells and has been applied to quantify a specific microorganism in biofilm, because is very useful to determine the number of RNA transcripts from bacterial biofilms. qRT-PCR have the advantage to be highly sensitive, and can be used to quantify gene expression from small amount of biofilm samples. SYBR Green and dual-labeled probe (Taqman) are the most

frequently used qRT-PCR methods and can to discriminate and count both live and dead cells in a microbiological sample (J Bueno, 2014; Roy et al., 2021).

B. Fluorescence in situ hybridization FISH

The multiplex fluorescence in situ hybridization (M-FISH) is a method that use fluorescent-labeled oligonucleotide probes specific 16S rRNA sequences and have allowed in situ analysis of the spatial and temporal dynamics of different bacterial populations within oral biofilms. The advantages of using M-FISH to spatially discriminate between various members of the microbial community involve the ability for identification of uncultured bacteria and the rapid manufacturing of new oligonucleotide probes, the combined use of M-FISH with CLSM monitors permits obtain three-dimensional spatial distribution of different bacteria in multispecies biofilms and can quantify semi planktonic biofilms in their natural habitat. FISH is a genetic alternative because can be applied to environmental and clinical samples, some authors have showed that FISH limitations can be solved with peptide nucleic acid (PNA) probes that using synthetic DNA analogues with stronger binding to DNA/RNA, and present higher specificity and sensitivity than conventional DNA probes (J Bueno, 2014; Dutta et al., 2021).

I.9.6. Microscopic techniques for biofilm analysis

In a method involving light microscopy, ascertain fluorescent dye that can be propidium iodide or a non-fluorescent dye like safranin can be used in staining the bacterial biofilm. If the resolution of the microscope is high enough, then the microbial cells can be counted. The staining of the biofilm and all the bio slime can be made possible by using dyes like alcian blue, which is able to bind to the glucose amino glycan and acidic mucopolysaccharides of the EPS. Now the stained bacterial biofilm appears blue or bluish green in colour under the microscope. Both scanning electron microscopy [SEM], field emission SEM [FESEM] and transmission electron microscopy [TEM] provide high-resolution images that help us to characterize microbial biofilms both structurally and morphologically. However, the data provided by these techniques are very in depth, yet the extensive sample preparation protocols, which include dehydration; fixation, freeze-drying, etc. make it a cumbersome process. Another disadvantage of these processes is that it, these treatments, affects the original biofilm morphologies deeply (Arunachalam & Davoodbasha, 2021; Roy et al., 2021).

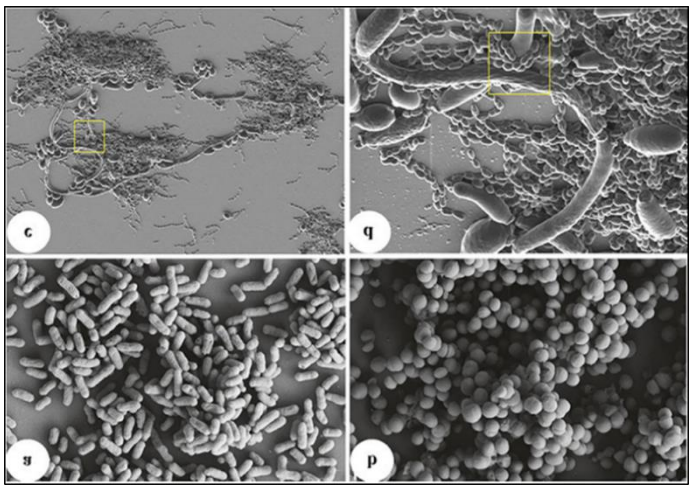


Figure 7: FESEM micrographs of E. coli and S. aureus

Figure 7: FESEM micrographs of *Escherichia coli* (10000X) (**a**) and *Staphylococcus aureus* (15000X) (**b**) grown at mono species conditions. Dual species biofilm of *Candida albicans* and *Streptococcus gordonii* (**c** and **d**, 1000 X and 5000 X , respectively) on FBS coated glass microscopic slides (Arunachalam & Davoodbasha, 2021).

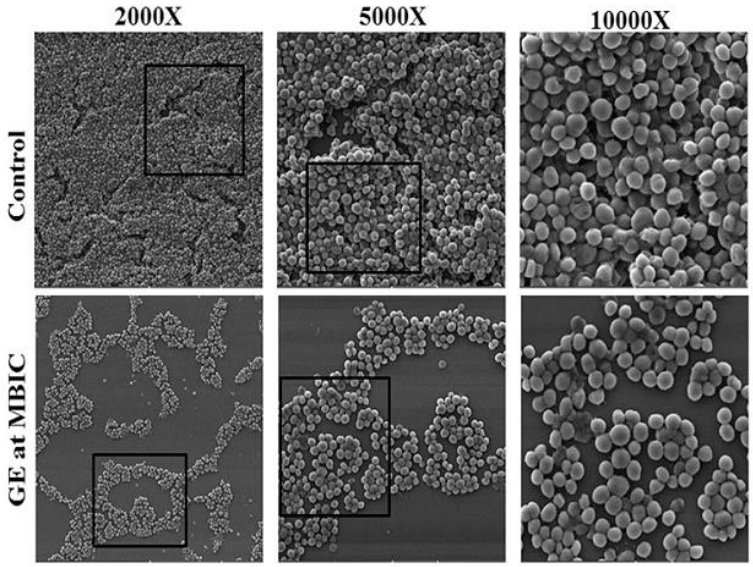


Figure 8: SEM obseration of biofilm formed by S. epidermidis

SEM observation of biofilms formed by *S. epidermidis* RP6 2a in glass slides in the presence and absence of geraniol (GE) at biofilm inhibitory concentration. Image representing multi layered biofilm formation of *S. epidermidis* covered with EPS in control panels. Upon treatment with geraniol, biofilm formation was reduce d, leaving discrete cell s at the bottom of the glass slides (Arunachalam & Davoodbasha, 2021).

I.10. Biofilm biological control :

It has been proposed that removing cells from the protective shield of a biofilm will render them more susceptible to antimicrobials and the host immune response (Silva et al., 2023). The current understanding about the mechanisms of dispersal and the broad phenotypes dispersed cells can adopt, one would not necessarily predict that dispersion would be an effective method for biofilm control (Gao et al., 2024). It is possible that dispersed cells would be more tolerant to antimicrobials than MIC testing on planktonic cells would predict, be highly virulent and adept at forming new biofilms at other locations. Thus, dispersing cells, without the capability of efficiently killing them, could

result in a substantially larger ecological problem or more deadly infection. However, despite these perils, dispersal agents have gained traction over the past decade as a viable therapeutic option, and many published studies have demonstrated proof of principal for this strategy (Rumbaugh & Sauer, 2020).

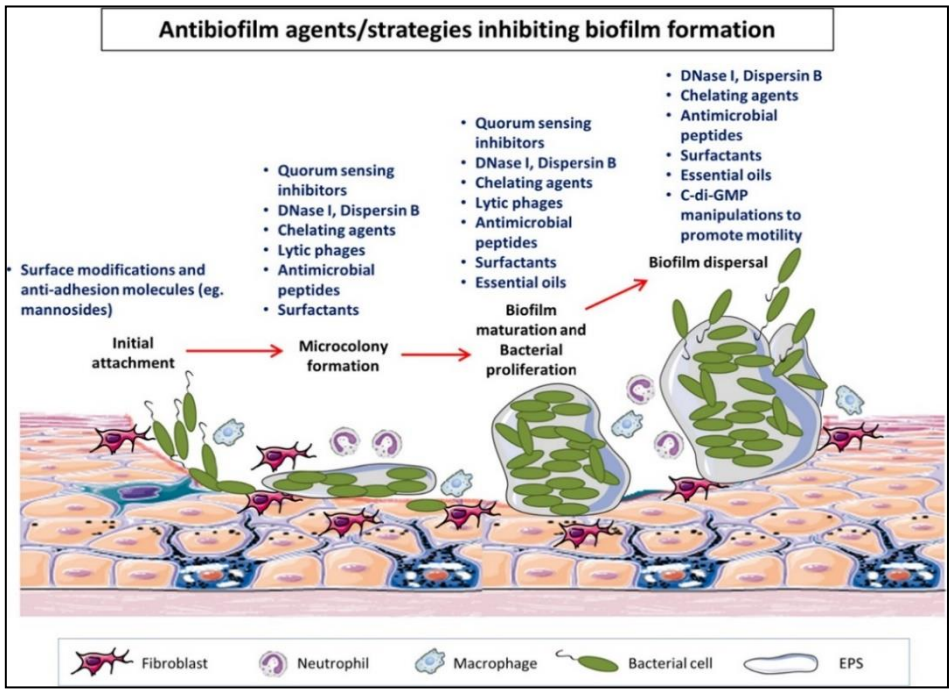


Figure 9: Biofilm formation on wound surface, anti-biofilm agent and anti-biofilm strategies

Biofilm formation on the wound surface, and anti-biofilm agents and strategies for biofilm inhibition and/or dispersal. C-di-GMP, cyclic dimeric guanosine monophosphate; EPS, extracellular polymeric substance (Razdan et al., 2022)

I.10.1. Nanotechnology:

In recent years, the use of nanotechnology has also emerged as a promising alternative strategy to treat biofilms. Nanotechnology is based on the use of molecules in the range of 1–1000 nm, the small size of which allows them to penetrate the biofilm layers. Nanotechnology research on biofilm treatments has focused on two main areas: the use of nanoparticles with antimicrobial activity and the development of drug delivery systems (Pinto et al., 2019). The first category includes the use of inorganic particles such as silver, zinc, titanium ,copper and gold (Juan Bueno & Bueno, 2020). The use of nanotechnology offers multiple advantages in comparison to traditional treatments. For instance, materials with greater surface area to volume ratios have improved reactivity. Furthermore, nanotechnology avoids problems such as enzymatic degradation, toxicity and unspecific delivery. Although there are diverse benefits deriving from the use of nanoparticles (Kranjec et al., 2021). Among all these remedies, nanoparticles have been used as promising candidates as anti-biofilm and anti-QS agents in preventing device-associated infections. Nanoparticles can be synthesized via physical, chemical, and biological methods (Arunachalam et al., 2023). It can be synthesized through a green route because they are less toxic and cost-effective. AgNPs exhibited significant inhibitory

activity in combination with antibiotics. Algae, bacteria, fungi, and plants are some biological agents used in nanoparticle synthesis (LewisOscar et al., 2021).

I.10.2. Quorum sensing:

QS is an intercellular chemical communication process in a cell-density dependent manner in which bacteria coordinate the expression of QS-mediated genes based on the exchange of small signaling molecules de fined as quorum sensors or autoinducers (AIs). Chemically, QS is based on the synthesis, sensing, and uptake of AIs (Kamath et al., 2023). Once a particular threshold concentration of bacteria is reached, programmed changes that coordinate biological effects including biofilm formation, virulence secretion, swarming ability, sporulation, and protease production are motivated in a density-dependent manner (Sionov & Steinberg, 2022). Several QS blocking strategies are directed to looking for inhibition of the synthase enzyme responsible for the production of the signalling molecule or receptor protein; inhibition of the chemical signal mediated by OHHL; or inhibition of the receptor protein that modulates quorum sensing. In this way enzyme and receptor-coupled high-throughput cell-free screen have been developed for find inhibitors of intercellular quorum sensing signals as quorum sensing inhibitors approach (J Bueno, 2014; Escobar-Muciño et al., 2022).

I.10.3. Enzymes as anti-biofilm agents:

Few recent investigations have shown the potential utilization of enzymes as anti-biofilm compound for the prevention or treatment of biofilm related infections. Different enzymes from prokaryotes, animals, and humans have the efficacy to degrade biofilm matrix or ECM. Targeting the QS process, which is a bacterial communication system (Lahiri et al., 2022). There are four different enzymes found in prokaryotes that are known to degrade AHLs (acylhomoserine lactone) in gram-negative bacteria (Mishra et al., 2020); those are AHL-lactonases, decarboxylases, AHL-acylases, and deaminases. AHL-acylases and lactonases degrade AHLs and disrupt the biofilm formed by *P. aeruginosa*. Acylase enzyme isolated from Aspergillus melleus is also used for coating the surface of a urinary catheter for its activity against P. aeruginosa biofilm formation (J. Yadav et al., 2021).

I.10.4. Antibiotics:

It is well known that antibiotic therapy is the most important and effective measure to control bacterial infection (Hutchings et al., 2019). However, bacterial biofilms are highly resistant to antibiotic treatment and immune response. Antibiotics have been widely used to treat biofilm infections, but clinical treatment still faces many challenges due to drug resistance issues, biofilm matrices that hinder drug penetration, and drug-microbe interactions. Therefore, many new anti-biofilm technologies have been developed, such as combining antibiotics and using new strategies, for

example, gallium, phage therapy, and antimicrobial photodynamic therapy (aPDT), to inhibit biofilm formation (Yin et al., 2022).

I.10.5. Antimicrobial peptides (AMPs):

Biofilm infection can be modulated through the use of antibiotics; however, antibiotics eliminate both pathogenic and commensal bacteria. In addition, due to their incomplete absorption by humans and animals, large amounts of ingested antibiotics are excreted into the environment via the faeces or urine, contributing to environmental and multi-drug resistance concerns. Therefore, new antimicrobial molecules are needed to effectively modulate microbial symbiosis to address these concerns (Huan et al., 2020).

Antimicrobial peptides (AMPs) are the small molecular peptides that play a crucial role in the innate immunity of the host against a broad range of microorganisms, including bacteria, fungi, parasites and viruses. To date, the AMP database [Data Repository of Antimicrobial Peptides (DRAMP)], http://dramp.cpu-bioinfor.org/] has reported 3791 AMPs from six kingdoms, including 431 from bacteria, 4 from archaea, 7 from protozoal, 6 from fungal, 824 from plants and 2519 from animals. Besides antibacterial activities, AMPs have been found to possess a variety of biological functions, such as immune regulation, angiogenesis, wound healing and antitumor activity (Zhang et al., 2021). Among AMPs produced by bacteria, bacteriocins, like nisin, which is produced by *Lactococcus lactis*, are active against both gram positive and gram-negative bacteria, including *S. aureus*, and *Listeria monocytogenes* (Mishra et al., 2020).

Among human AMPs, LL-37 is expressed by several immune and epithelial cells and is directly involved in the host cellular response to microbial attacks. LL-37 has anti-fungal, antimicrobial and anti-biofilm properties can act as a chemoattractant for human peripheral blood neutrophils, monocytes, and T-cells, and is even capable of inhibiting Kaposi's sarcoma-associated-herpesvirus. (Huan et al., 2020; Radaic & Kapila, 2021).

II. Generalities on Actinobacteria genius

II.1. Classification of Actinobacteria:

Actinobacteria are Gram-positive bacteria that grow in a variety of environments, they can live in a variety of environments and are widely distributed in the natural ecosystems (.e.g. soil, rhizosphere, marine, sediments…etc.) (Xie & Pathom-Aree, 2021). The Actinobacteria distinguish themselves morphologically by forming a layer of hyphae that carry chains of spores known as aerobic filamentous Actinobacteria and further reproduce by sporulation many Actinobacteria can produce mycelium (Muazi Alenazi et al., 2023).

Actinobacteria are well recognized biosynthetic factory that produce an abundant secondary bioactive metabolites, such as antibiotics, anticancer drugs, immunosuppressive drugs, enzymes, enzyme inhibitors, and other therapeutic or biologically active compounds like anti-biofilm which scientist look for (Jose et al., 2021).

II.1.1. The phylum Actinobacteria:

Actinobacteria is a phylum of Gram-positive bacteria, and members of bacteria belonging to this phylum are classified into 6 classes, 46 orders, 79 families, 404 genera and 3000 (N. Salam et al., 2020), the classes are namely *Acidimicrobiia*, *Actinobacteria*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria* and *Thermoleophilia* **Fig. 10**, . Among the six different classes, members of the Actinobacteria class are the most dominant and contain one of the largest genera, *Streptomyces*, with higher than 961 distinct species. Members of phylum Actinobacteria are omnipresent, and have been isolated from various extreme environments (high temperatures, pH, salinities, pressure and drought), and are specially found in rhizosphere soil. Based on literature analysis members of phylum Actinobacteria have been reported from different genera such as *Acidimicrobium*, *Antinomies*, *Arthrobacter*, *Bifidobacterium*, *Cellulomonas*, *Clavibacter*, *Corynebacterium*, *Frankia*, *Microbactrium*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, *Pseudonocardia*, *Rhodococcus*, *Sanguibacter*, and *Streptomyces* (A. N. Yadav et al., 2018).

Besides, and based on molecular taxonomy, the phylum “Actinobacteria” is well supported by analyses of the 16S and 23S rRNA genes, presence of conserved insertions and deletions (or indels) in certain proteins, and characteristic gene rearrangements (Goodfellow & Fiedler, 2010).

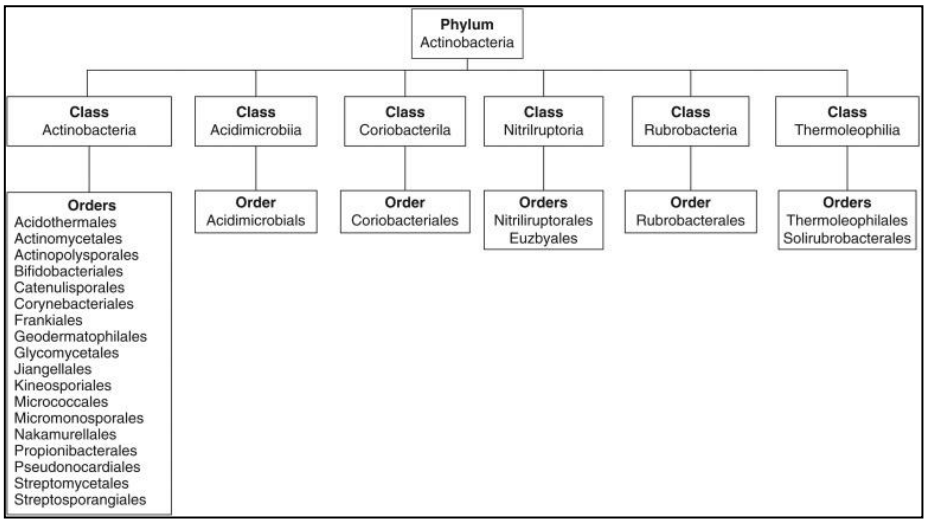


Figure 10: Taxonomy of the phylum Actinobacteria (Lawson, 2018)

II.1.2. Class Actinobacteria:

Based on data genome, the phylogeny of the class Actinobacteria remains controversial, the orders Bifidobacteriales, Coriobacteriales, 'Corynebacteriales', 'Micromonosporales', 'Propionibacteriales', 'Pseudonocardiales', Streptomycetales, 'Streptosporangiales' 'Frankiales' and 'Micrococcales' were recovered in phylogenetic tree **Fig. 11**. However, they also registered as a sub order Frankineae and Micrococcineae under the order Actinomycetal. It is thus proposed that the order 'Frankiales', be split into Frankiales ord. nov. (Type family Frankiaceae), Geodermatophilales ord. nov. (Geodermatophilaceae), Acidothermales ord. nov. (Acidothermaceae) and Nakamurellales ord. nov. (Nakamurellaceae).The order Micrococcales should also be split into Micrococcales (genera Kocuria, Rothia, Micrococcus, Arthrobacter, Tropheryma, Microbacterium, Leifsonia and Clavibacter), Cellulomonales (Beutenbergia, Cellulomonas, Xylanimonas, Jonesia and Sanguibacter) and Brachybacteriales but the formal proposal is not yet available for a significant proposal (Lawson, 2018).

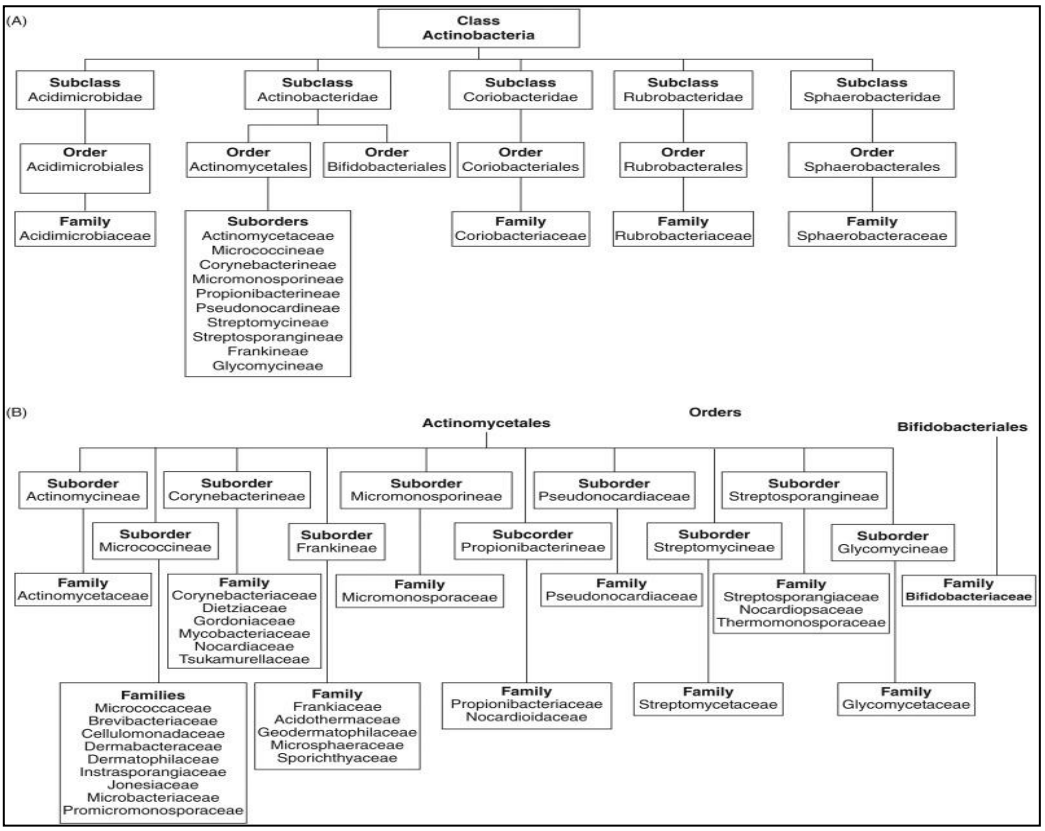


Figure 11: Taxonomy of the class Actinobacteria and their subclasses (Lawson, 2018)

II.2. Colonies morphology, mycelium structure and spores:

Actinobacteria exhibit a wide variety of morphologies at macroscopic and microscopic levels. In the case of the filamentous species belonging to *Actinomyces* or *Streptomyces genus,* firstly colonies are relatively smooth surfaced (Peñil Cobo et al., 2018) but later they develop a weft of aerial mycelium that may appear floccose, granular, powdery, or velvety. They produce a wide variety of pigments responsible for the colour of the vegetative and aerial mycelia (Cordovez et al., 2015).

Actinobacteria group, including coccoid (*Micrococcus*) and rod-coccoid *(Arthrobacter*), as well as fragmenting hyphal forms (*Nocardia spp*.) and also forms with permanent and highly differentiated branched mycelia (e.g., *Streptomyces spp*., *Frankia*), Rhodococci form elongated filaments on the substrate and do not produce a true mycelium, while corynebacteria do not produce mycelia at all. However, as in other Actinobacteria, the filaments grow at the apex instead of by lateral wall extension. Actinobacteria belonging to the genus *Oerskovia* are characterized by the formation of branched substrate hyphae that break up into flagellated motile elements Further, mycobacteria and rhodococci do not usually form aerial hyphae, although some exceptions exist (Barka et al., 2016a)

Spore Chain Morphology With relevancy to store chains, the strains are sorted into „sections". The species belonging to the genus Streptomyces are divided into three sections, particularly rectiflexibiles, retinaculiaperti and Spirales. Once a strain forms two types of spore chains, both are noted (M. Sharma et al., 2014).

Streptomyces species have chains of spores on the aerial mycelium, which are normally absent from the substrate mycelium. These spores are arthrospores, regular segments of hyphae with a thickened spore wall surrounded by a hydrophobic sheath that may bear spin or hairs (Dilip et al., 2013).

Spores are extremely important in the taxonomy of Actinobacteria. The initial steps of sporulation in several oligosporic Actinobacteria can be regarded as budding processes, because they satisfy the main criteria used to define budding in other bacteria. Spores may be formed on the substrate and/or the aerial mycelium as single cells or in chains of different lengths. In other cases, spores may be harbored in special vesicles (sporangia), and endowed with flagella as described in **fig. 12** (Barka et al., 2016a).

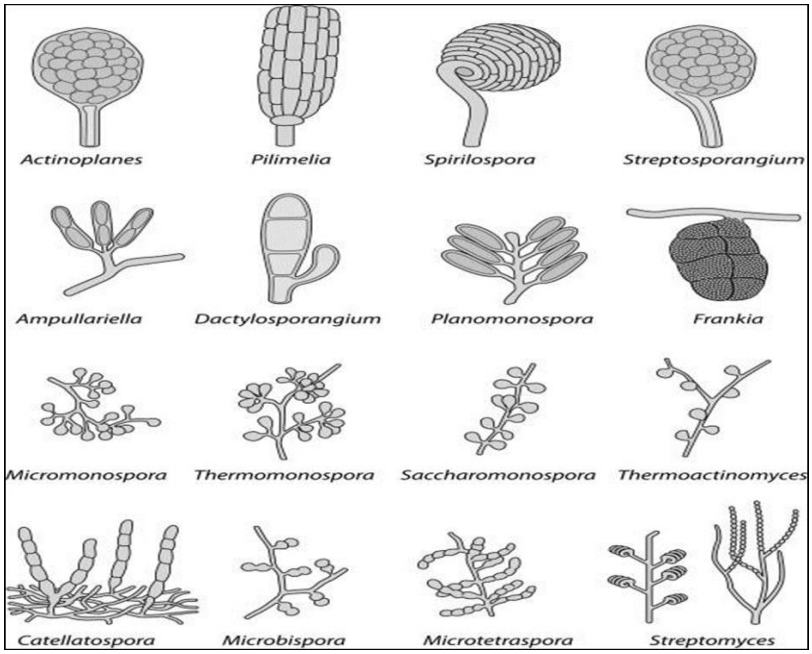


Figure 12:Schema of the different types of spore chains produced by filamentous Actinobacteria (Barka et al., 2016a)

II.3. Biology of Actinobacteria : physiology and nutritional metabolism

Filamentous Actinobacteria are heterotrophic in nature, most of them are strict saprophytes, while some from parasitic or mutualistic associations with plants and animals. Actinobacteria are commonly believed to have a role in the recycling of nutrients. They are aerobic and some like Actinobacteria are anaerobic (A. N. Yadav et al., 2018). The species like Frankia require very specialized growth media and incubation conditions. Many Actinobacteria are growing on the common bacteriological media used in the laboratory such as nutrient agar, trypticase agar, blood agar, brain heart infusion agar and starch casein agar. *Sporoactinomyces* require special media to allow differentiation and the development of characteristic spores and pigments. Some of these media are not available commercially and must be prepared in the laboratory (Dilip et al., 2013).

Soil-dwelling organisms that spend the majority of their life cycles as semi-dormant spores, especially under nutrient limited conditions. However, the phylum has adapted to a wide range of ecological environments: Actinobacteria are also present in soils, fresh and salt water, and the air. They are more

abundant in soils than other media, especially in alkaline soils and soils rich in organic matter, where they constitute an important part of the microbial population. Actinobacteria can be found both on the soil surface and at depths of more than two meters below ground.

The population density of Actinobacteria depends on their habitat and the prevailing climate conditions. They are typically present at densities approximately 10^6 to 10^9 cells per gram of soil; soil populations are dominated by the genus *Streptomyces*, which accounts for over 95% of the Actinobacteriales strains isolated from soil. Other factors, such as temperature, pH, and soil moisture, also influence the growth of Actinobacteria. Like other soil bacteria, *Actinobacteria* are mostly mesophilic, with optimal growth at temperatures between 25 and 30°C. However, thermophilic *Actinobacteria* can grow at temperatures ranging from 50 to 60°C. Vegetative growth of Actinobacteria in the soil is favored by low humidity, especially when the spores are submerged in water. In dry soils where the moisture tension is greater, growth is very limited and may be halted. Most Actinobacteria grow in soils with a neutral pH. They grow best at a pH between six and nine, with maximum growth around neutrality. However, a few strains of Streptomyces have been isolated from acidic soils (pH 3.5) (Barka et al., 2016a).

II.4. Actinobacteria in rhizosphere microbiome:

The rhizosphere is an area of intensive interaction among plant roots, microorganisms and soil. The rhizosphere **fig.13** is of central importance for microorganism-driven carbon sequestration, ecosystem functioning *and* nutrient cycling *in* terrestrial ecosystem. Therefore, the microbial richness is much greater in rhizosphere area than bulk soil. Further, in terms of the functional potential of microbial community, the activity of enzymes in the rhizosphere is much higher than bulk soil. For example, 5777 genes (93.2% of total 6201 genes) were detected in the rhizosphere, while only 1983 genes (32.0%) were detected in the bulk soils, confirming the functional superiority of rhizosphere for microbial processes. In the rhizosphere part, 5390, 103 and 246 genes were from bacteria, archaea, and fungi respectively whereas that was 1849, 38 and 84 in bulk soil. It was interesting that 53 gene families (out of 248) were detected only in the rhizosphere. This highlights the greater richness of species and their functions in the rhizosphere soil, than bulk soil (Jansson & Hofmockel, 2018).

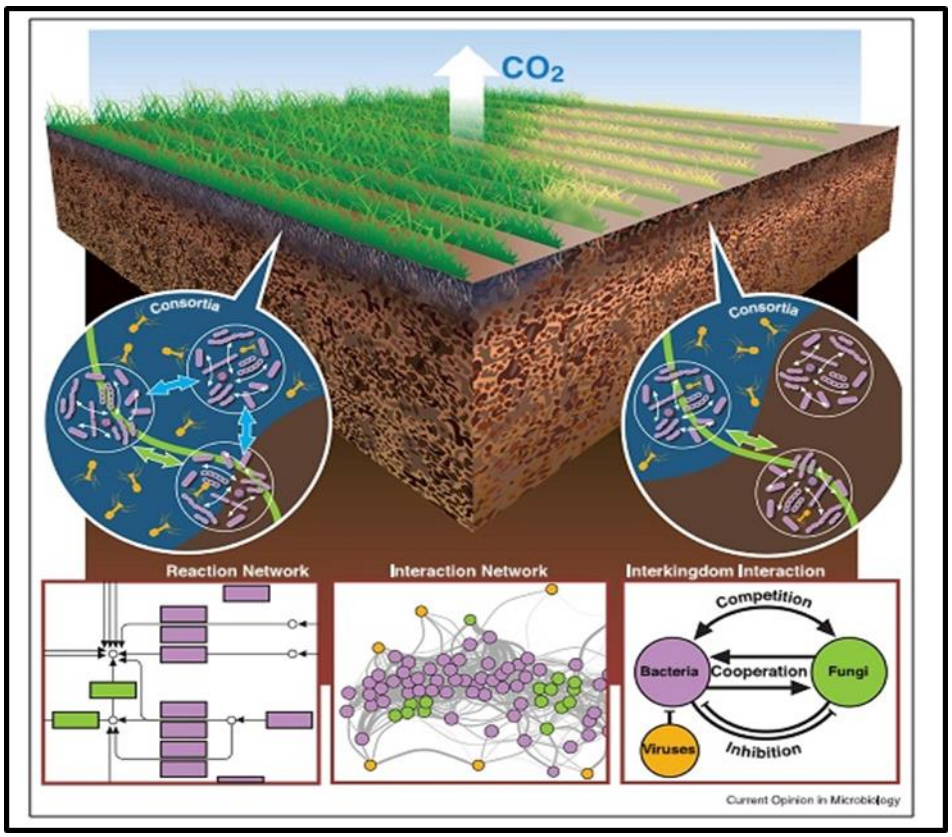


Figure 13:Oerview of soil microbial community interaction (Jansson & Hofmockel, 2018).

The microbes in rhizosphere help plants in growth-promotion and yield. Actinobacteria are one of the major components of rhizosphere microbial populations and are useful in soil nutrient cycling as well as Plant Growth-Promotion (PGP). Actinobacteria produce secondary metabolites such as lytic enzymes, PGP substances and antibiotics. The Actinobacteria, mainly those belonging *to Streptomyces* sp., makeup an important group of soil microbes. Streptomyces are abundant in soil and help in the degradation of complex molecules to simple molecules for plant growth and development. These are also reported to decompose organic matter, promote plant growth and control plant pathogens (Sreevidya et al., 2016).

II.5. Actinobacteria as promising natural source of antibiotics and anti-biofilm agents

Worldwide, the infectious diseases and multidrug resistance have always been challenging global health (Aslam et al., 2018; Mary et al., 2021). Therefore, the discovery of novel bioactive molecules with innovative mechanism of action create a promising solution in the design of alternative therapeutic solution. Consequently, researchers have been constantly looking all kind environments for new sources of novel bioactive compounds (Miethke et al., 2021; Prestinaci et al., 2015; Quinn et al., 2020). Microbiologists have done many studies on the isolation and screening of antimicrobial producing *Streptomyces* (Bouras et al., 2021; Meklat et al., 2020). It has been recorded that the most of new antimicrobial molecules are come from the screened soil isolates (Djinni et al., 2019; Quinn et al., 2020; Reggani et al., 2021; Sapkota et al., 2020). Species affiliated with *Streptomyces* genus

are Gram positive with higher GC% and, are ubiquitous in the environment (Barka et al., 2016b; Cordovez et al., 2015). Most of them are aerobic, saprophytic microorganisms with complex life cycle, they are capable of both solitary inhabitation and forming symbiosis not only with microorganisms but also with higher organisms (Prudence et al., 2020).

Actinobacteria class strains have been reported as bountiful producers of secondary metabolites with several significant biological activity, the filamentous spaces are considered as the most economical and biotechnological important microorganisms (Azman et al., 2019; Gunjal & Bhagat, 2022). Actinobacteria contributing around70% of bioactive compound, 80% are produced by *Streptomyces*, while 20% are isolated from non-*Streptomyces* (Law et al., 2020; Prudence et al., 2020). *Streptomyces* species belonging to the rhizosphere soil microbial communities and are efficient colonizer of different plant compartments from the root to the areal parts. In fact, they are active producers of antibiotic and organic volatile compounds, both in soil and in plants, and this features is helpful to identifying active antagonist of pathogen and can be used in several medicine sectors as biocontrol agents (Kamil et al., 2018; Vurukonda et al., 2018).

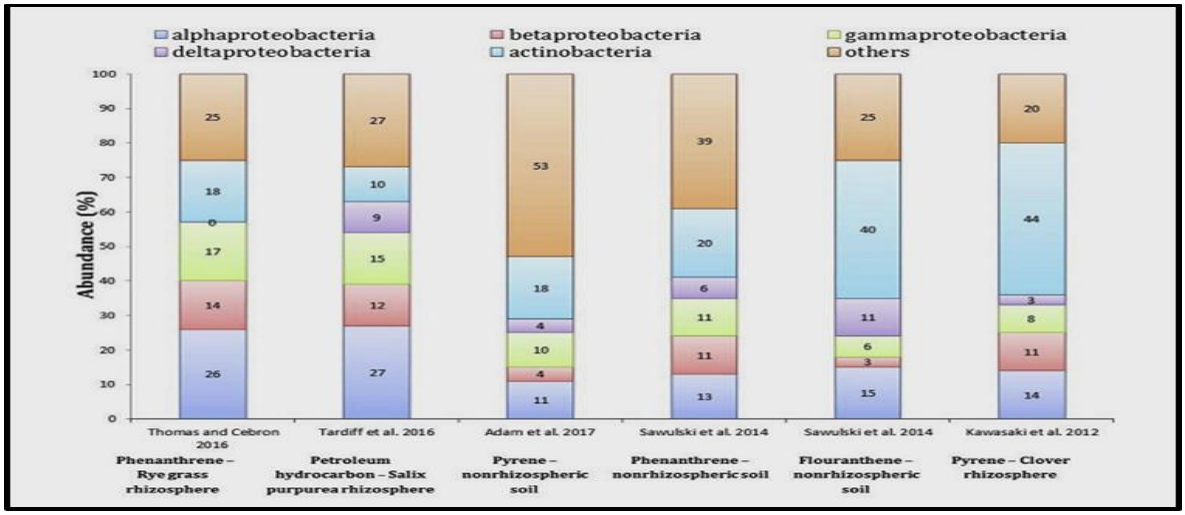


Figure 14: Percentage of Actinobacteria in soil microbiome reported in hydrocarbon rhizospheric/ non rhizospheric (Kotoky et al., 2018)

Endophytic Actinobacteria (EA) that coexist with medicinal plants residing within their robust tissues have very beneficial and important effects on the survival and life of their hosts, which are mostly unknown. Plants harbour novel and diverse range of Actinobacteria, and have always been considered as one of the new untapped sources for isolation of EA. Isolation of Actinobacteria strains from different environments will probably lead to the identification of new species with high ability to produce bioactive compounds. Accordingly, the isolation and identification of Actinobacteria have recently become a productive area of research that has consequently led to the identification of novel Actinobacteria species that need to be exploited to unveil possible biosynthetic pathways and discover new bioactive natural metabolites (Delbari et al., 2023).

Studies have shown that most of Streptomyces species exhibits anti-biofilm activities. Recently, the ethyl acetate extract from Streptomyces sp. SBT343 was found to significantly inhibit *Staphylococcus epidermidis* RP 62A biofilm formation on polystyrene, glass, and contact lens surfaces, without affecting bacterial growth (Balasubramanian et al., 2017). The ethyl acetate extract also displayed similar antagonistic effects to-wards the formation of S. aureus biofilms but had no inhibitory effects against Pseudomonas biofilms. The formation of S. epidermidis biofilm is facilitated by the synthesis of the homo polymer polysaccharide intercellular adhesion (PIA). In certain condition, S. epidermidis can switch between PIA-dependent and PIA-independent modes of biofilms lifestyles (Hennig et al., 2007). The presence of the ethyl acetate extract of Streptomyces sp. SBT343 , however, prevented the switching of the PIA-dependent and PIA-independent biofilm lifestyles (Balasubramanian et al., 2017). Meanwhile, another study on the culture supernatant of Actinobacteria species strains SW19, KP12, and CW17, isolated from lake, river, and paddy field had demonstrated high anti-biofilm activities against pathogenic biofilms such as *P. aeruginosa* ,enterotoxigenic *E. coli* , *Vibrio parahaemoluticus* , *Vibrio cholera* , *Streptococcus pneumonia* , *Staphylococcus aureus* ,and *Enterococcus faecalis*. Further characterization showed the bioactive compounds consisted of nucleic acid, protein, and polysaccharides where they interrupt the cell surface and interaction between cells, which is a requirement for biofilm development (Waturangi et al., 2016). Polysaccharides can produce anti-adherence effects between microorganisms and surfaces, while extracellular DNA is able to bind to the adhesive structure of the planktonic cells needed for attachment to the surface. The protein (extracellular enzyme) produced by *Streptomyces sp.* is also able to interfere with extra cytoplasmic proteins (e. g., surface exposed proteins), which plays a role in bacterial attachment to abiotic surfaces (Rendueles et al., 2013; Sayem et al., 2011).

Three peptidic metabolites designated as cahuitamycins A, B, and C were discovered from *Streptomyces gandocaensis* strain DHS334, and it was found that only cahuitamycin C showed highly effective inhibition effects on the biofilm formation of *Acinetobacter baumannii*. Further experiment through selective mutasynthesis o f *S. gandocaensis* strain DHS334 had led to the isolation of two unnatural analogues: cahuitamycins D and E. When subjected to static biofilm assays, the analogue cahuitamycin D demonstrated twofold enhanced inhibitory activities against *A. baumannii* biofilm as compared to cahuitamycins C. Findings from this study had suggested that the use of genetic engineering on Actinobacteria strains may represent a favourable alternative for discovering and developing new therapeutics against biofilms (Park et al., 2016). On the other hand, streptorub in B extracted from *Streptomyces sp*. M C11024 which

belongs to the prodiginine group of antibiotics was able to inhibit the biofilm formation of methicillin resistance S. aureus (MRSA) N315 (Suzuki et al., 2015). Prodiginines are gaining much attention as they have been shown to exhibit various bioactivities including antimalarial and antibacterial agents. Generally, prodiginines induce oxidative DNA cleavage and chelate metals and thus, it was suggested

that the inhibitory effects of streptorubin B on biofilm formation maybe due to these abilities. Although the mode of action of streptorubin B is still unclear, it remains a good candidate to be developed as biofilm formation inhibitors of *S. aureus* (Suzuki et al., 2015).

Table 1: Recapitulate studies on Actinobacteria sp, and their mode of action against biofilm formation

Actinobacteria strains	Anti-biofilm mode of action	Reference
Streptomyces albus A66 extract	Reduces biofilm formation and disperses mature biofilm; inhibits quorum sensing system	(You et al., 2007)
S. akiyoshiensis A3 extract	Inhibits biofilm formation; reduces cell surface hydrophobicity	(Thenmozhi et al., 2009)
S. akiyoshiensis CAA-3 methanolic extract	Reduces biofilm formation	(Bakkiyaraj & Karutha Pandian, 2010)
Arctic Streptomyces sp. A731, Nocardiopsis sp. A733, Streptomyces sp. A745	Reduces biofilm formation	(Augustine et al., 2012)
Extracellular peptides/proteins from Streptomyces sp. BFI230	Inhibits biofilm formation without affecting planktonic growth; interferes with iron acquisition process	(Kim et al., 2012)
Streptorubin B from Streptomyces sp. MC11024	Inhibits biofilm formation without affecting cell growth; may induce oxidative DNA cleavage and chelate metals	(Suzuki et al., 2015)
Actinobacteria SW19, KP12, and CW17	Inhibits biofilm formation; interferes and interrupts the cell surface and cell-cell interactions	(Waturangi et al., 2016)
Extract from Actinobacteria strain Streptomyces sp. SBT343 organic extract	Inhibits biofilm formation without affecting growth; interferes with PIA-mediated biofilm formation	(Balasubramanian et al., 2017)
Nocardiopsis sp. GRG 1 (KT235640)	Antibiofilm effect of Nocardiopsis sp. GRG 1 (KT235640) compound against biofilm forming Gram negative bacteria on UTIs	(Rajivgandhi et al., 2018)
Streptomyces griseoincarnatus strain HK12	Fatty acyl compounds from marine Streptomyces griseoincarnatus strain HK12 against two major bio-film forming nosocomial pathogens; an in vitro and in silico approach	(Kamarudheen & Rao, 2019)
Streptomyces californicus Strain ADR1	Isolation and Characterization of a New Endophytic Actinobacteria Streptomyces californicus Strain ADR1 as a Promising Source of Anti-Bacterial, Anti-Biofilm and Antioxidant Metabolites	(R. Singh & Dubey, 2020)
Streptomyces W-5A	The Activities of Streptomyces W-5A as antibacterial and antibiofilm towards methicillin-resistant Staphylococcus aureus 2983	(Dinda et al., 2021)
Streptomyces W-5B	The Production of Streptomyces W-5B Extract for Antibiofilm against Methicillin-resistant Staphylococcus aureus	(Asnani et al., 2022)
actinobacteria Amycolatopsis sp. KMN	Antibiofilm and cytotoxic potential of extracellular biosynthesized gold nanoparticles using actinobacteria Amycolatopsis sp. KMN	(Kabiri et al., 2023)
actinobacterium Glutamicibacter uratoxydans VRAK 24	Evaluation of heavy metal removal and antibiofilm efficiency of biologically synthesized chitosan- silver Nano-bio composite by a soil Actinobacteria Glutamicibacter uratoxydans VRAK 24	(Vishnupriya et al., 2024)

To date, significant progresses on understanding biofilm formation processing environments have been achieved as microbial environmental sensing, and factor interactions have been fully elucidated, and most of the biofilm studies adopted a reductionist approach, trying to oversimplify complex

ecological systems, using a powerful design of experiment, and High-throughput methodologies for the study of mono- and multi-species biofilm model systems.

CHAPTER TWO: *MATERIAL AND METHODS.*

1. Isolation of pathogenic clinical strains

1.1. Collection of clinical samples, Isolation and identification of pathogenic strains:

The 30 clinical samples were collected from clinical samples of patients undergoing treatment in MESBAH BAGHDAD hospital, Tamanrasset, Algeria. The clinical samples received in the laboratory including, pus, urine, and tissues were examined. From all clinical samples processed during study period, the researched strains were identified following standard microbiological procedures (Cheesebrough, 1998). Firstly, the clinical isolates were identified as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* strain on the basis of colony morphology on different selective culture media: Chapman, Hektoen and HiChrom from HiMedia, , Gram's stain, and different biochemical tests (Gallery API) (Bergey, 1994). Then the isolated strains were subjected to specifics confirmed identification, which performed using Matrix Assisted Laser Desorption Ionisation-Time Of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) (Calderaro & Chezzi, 2024; Lay Jr, 2001) realized in laboratory of «Klinik für Infektionskrankheiten und Spitalhygiene, Universitätsspital Zürich, Switzerland», December; 2023.

MALDI-TOF MS is an conventional analytical technique in which particles are ionized, separated according to their mass-to-charge ratio, and measured by determining the time it takes for the ions to travel to a detector at the end of a time-of-flight tube. The resulting spectrum, with mass-to-charge values along the x-axis and intensity along the y-axis, is compared to a database of spectra from known organisms. This technology demonstrating high reliability and effectiveness in this application, it can identify gram-positive, gram-negative, aerobic and anaerobic bacteria as well as mycobacteria, yeast, and molds, typically at the species level (Rychert, 2019).

1.2. Antimicrobial susceptibility testing and detection of β-lactamase:

The antimicrobial susceptibility profiles of bacterial isolates was released by the BD Phoenix™ (Becton Dickinson, USA) automated system realized in laboratory of «Klinik für Infektionskrankheiten und Spitalhygiene, Universitätsspital Zürich, Switzerland», December; 2023 (Carroll et al., 2006; Yuceel-Timur et al., 2024). Minimum inhibitory concentrations (MICs, µg/ml) were interpreted as susceptible (S), intermediate (I), or resistant (R). MDR isolates were defined phenotypically as those clinical isolates resistant to ≥3 antibiotic classes (Lob et al., 2021).

The BD Phoenix™ Automated Microbiology System is intended for in vitro quantitative determination of antimicrobial susceptibility by minimal inhibitory concentration (MIC) of most Gram-negative aerobic and facultative anaerobic bacterial isolates from pure culture for most Gram-positive bacteria isolates from pure culture. The system is comprised of disposable panels, which

combine both identification testing (ID) and antimicrobial susceptibility testing (AST), and an instrument which performs automatic reading at 20-min intervals during incubation. The system claims to provide accurate and rapid susceptibility results with easy workflow for the laboratory worker. The Phoenix AST method is a broth based microdilution test. The Phoenix panel is a sealed and self-inoculating molded polystyrene tray, with 136 micro-wells containing dried reagents. The ID/AST combination panel includes an ID side (51 wells) with dried substrates for bacterial identification and an AST side (85 wells). The AST panel contains a wide range of two-fold doubling dilution concentrations of antimicrobial agents and growth and fluorescent controls at appropriate well locations. The AST panel does not include wells for isolate identification. The Phoenix System utilizes a redox indicator for the detection of organism growth in the presence of an antimicrobial agent (Fahr et al., 2003; Gajic et al., 2022; M. A. Salam et al., 2023).

2. Biofilm Detection Assays:

2.1. Qualitative analysis of biofilm production:

2.1.1. Congo red assays (CRA)

The biofilm qualitative assays was performed on Congo Red Agar (CRA) medium (C. J. Jones & Wozniak, 2017), which constructed by mixing 0.8 g/L g of Congo red, 36 g/L of sucrose, 37 g/L of Brain heart infusion broth (BHIB) all from CHEMESTERY and 15 g/L of agar. After incubation period that was 24 h at 37°C, morphology of colonies that undergone to different colors is differentiated as biofilm producers or not. Black to gray colonies with a dry crystalline consistency indicate biofilm producers, whereas colonies retained pink or red are non-biofilm producers (Jebril, 2020; Kaiser et al., 2013).

2.1.2. Tube adherence method assays (TAM):

The isolated clinical bacteria were inoculated in 5 ml Brain Heart Infusion Broth (BHIB), as typical medium to form biofilm as recorded by Lopes and collaborators (Lopes et al., 2023; A. K. Singh et al., 2017). In test tubes and incubated at 37 °C/ 24h, after the incubation the tubes were decanted , and dried for 10min then stained with 0.1% Cristal Violet for 15nim. Afterward, the tubes were washed genteelly then placed upside down for drying. Visible lining of the well and bottom of the tube by a film was considered as positive, the results was investigated visually as weak, moderate or strong biofilm producers as demonstrated by (Neopane et al., 2018).

2.2. Quantitative analysis of biofilm production:

2.2.1 Tissue culture plate method (TCP):

a) Initial inoculum, media, and incubation:

Briefly, the clinical isolates were grown on BHI agar overnight at 37°C. Then, a loopful colonies from overnight grown BHI agar from the culture plates were suspended directly into 5 ml of

physiological saline (0.89% NaCl), and vortexed to achieve a suspension of 0.5- McFarland turbidity (1.5×10^8CFU/ml) which is equivalent to (0.5-0.8 DO_{660}) (Haque et al., 2021). Individual wells of 96 well–flat bottom polystyrene (TRUST LAB, Ningbo, China) were filled with 180 µl aliquots of BHI and 20 µl of bacterial suspension was added to it. Then, the plates were read after incubation 37°C /24h (Obaid, 2019).

b) Fixation:

After respective incubations, the plates were inverted, and gently tapped to remove residual broth. The wells were washed thrice with 200 µl of phosphate buffer saline (PBS) (pH 7.2) to remove planktonic bacteria before fixation. The plates were then inverted and blotted on paper towels and allowed to air dry for 15 min The cells were fixed with 200 µl of sodium acetate (2% w/v) for 30 min (Ghellai et al., 2014; Obaid, 2019).

c) Staining and elution:

For staining, we used 200 µL of 0.5% crystal violet for 15 min. The excess crystal violet was removed, and the plates were washed with running tap water until runoff was clear. For elution, we used 200µl ethanol (95%) and left at room temperature for 30 min. The elute was then recuperated in wells of new TCP to take optical density (OD) readings at λ max 630 nm (Ghellai et al., 2014; Obaid, 2019) in an enzyme-linked immunosorbent assay reader (ELISA) plate reader (Bio-Tek Instruments, USA) (Harika et al., 2020).

d) Results investigation:

Spectrophotometric measurement of optical densities (OD) of adherent cells enabled us to classify our clinical isolates collection into four categories non adherent (OD ≤0.2), weakly (0.2<OD≤0.4), moderately (0.4<OD≤0.8) and strongly (0.8<OD) adherent strains The experiment was repeated three times separately for each strain and the average values were calculated with standard deviation (SD). To correct background staining, the OD values of the eight control wells were averaged and subtracted from the mean OD value obtained for each strain (Ghellai et al., 2014).

3. The soil Actinobacteria and anti-biofilm control:

3.1. Soil sample collection:

Seven soil samples were collected from seven variable region from Tamanrasset village, southern of Algeria, in October 2021 to selective isolation of Actinobacteria sp.; strains. The soil samples were taken from stable depth after the first 5cm of the rhizosphere zone of the dominating plants *Acacia Senegal* tree. The soil sample were placed in sterile plastic zip bags, and were aseptically transported to the research laboratory of University of Tamanrasset for further microbiological analysis. The sits and climate information of the isolation period were described in (**fig. 15, tab. 2**)

Table 2: Soil sampling procedure

Site	Location	Soil ecosystem	GPS	Altitude (Km)	Sampling date	T° min	T° max	T° m	Precipitation
1	ABALESSA	Acacia rhizosphere soil	22° 54' 02"N 4° 51' 55"E	0.891	01/10/2021	19,9	33,7	26,8	0,0
2	TIT	Acacia rhizosphere soil	22° 58' 04"N 5° 13' 17"E	1.12	03/10/2021	16,7	31,6	24,15	0,0
3	OUTOUL	Acacia rhizosphere soil	22° 51' 16"N 5°20 '40"E	1.29	04/10/2021	17,8	31,8	24,8	0,0
4	AGUENAR	Acacia rhizosphere soil	22° 50' 28"N 5°26 '58"E	1.38	08/10/2021	16,4	32,4	24,4	0,0
5	INZAOUAN	Acacia rhizosphere soil	22° 45' 49"N 4° 51' 55"E	1.38	11/10/2021	16,0	33,8	24,9	0,0
6	ADERIAN	Acacia rhizosphere soil	22° 47' 14"N 5°33 '24"E	1.42	15/10/2021	14,7	33,2	23,95	0,0
7	AHEGGAR	Acacia rhizosphere soil	22° 51' 08"N 5°34 '30"E	1.45	19/10/2021	14,0	31,7	22,85	0,0

Figure 15: Acacia Senegal tree

3.2. Actinobacteria Isolation:

As a sample pretreatment, a total of 5 g sieved soil sample was placed in glass petri dishes, and then heated in a Pasteur oven at 110 °C for 10 minutes until dried. Each samples were suspended in 45 mL distilled sterile water and subsequently serially decimal diluted up to 10^{-6}. From each dilution, 1 mL suspension was inoculated by standard spread method on Glycerol yeast extract agar (GYE) media (g/l: 1; K_2HPO_4, 5; glycerol, 2; yeast extract, 20 agar, pH: 7.6) and the plates were incubated at 31 °C

for 2 weeks. Colonies produced from each serially diluted plate were purified on the same culture media, and maintained in glycerol 30% (v/v) (Abussaud et al., 2013; Reggani et al., 2021).

3.3. Identification, screening and evaluation of antagonistic activity of Actinobacteria sp., isolates:

3.3.1. Morphological characterization:

Morphological characteristics of the isolates were determined with naked eyes as per the guidelines of international Streptomyces project (Shirling & Gottlieb, 1966). The growth pattern, colour of aerial mycelium, substrate mycelium, and diffusible pigments of isolated Actinobacteria were examined. Pure isolates were streak plated onto various media (GYE) and cultured for 7–14 days at 31°C. Gram staining procedures were used to examine macro-morphology were observed under light microscope oil immersion (100)x[10] (Barka et al., 2016b).

3.3.2. Screening of Actinobacteria strains:

The determination of antagonistic activities of the pure Actinobacteria cultures against the isolated clinical bacteria was performed by using cross-streak method on GYE media using cross streak method as described by (Balouiri et al., 2016; Velho-Pereira & Kamat, 2011), the inhibition zone were measured and registered.

The preparation and standardization of the bacterial Inoculum was realized following the method of Elias with some modification. Isolated colonies of test clinical pathogens from fresh culture were transferred to test tubes, containing sterile physiological water (10%), and used to match turbidity equivalent to 0.5 McFarland standards, which are equivalent to a cell density of 10^6–10^8 CFU/ mL for bacteria (F. Elias et al., 2022).

3.3.3. Molecular identification:

Molecular identification was carried out based on the sequencing of 16s rRNA. The Actinobacteria strains were cultured in GYE medium until the first appearance of the colony (Messaoudi et al., 2020). Extraction of genomic DNA, PCR amplification and direct sequencing of the PCR products of the 24 selected thermos-tolerant Actinobacteria were carried out in biotechnology centre of GENE LIFE SCIENCE, Sidi Bel Abbes 22002 Algeria. Following the below methodology:

a) DNA extraction, amplification and sequencing:

The extraction of bacterial genomic DNA was carried by using the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn Bhd, Selangor, DE, Malaysia) allowing to manufacturer's references. The effectiveness of this extraction procedure was verified using agarose gel electrophoresis. The selected 16S rRNA gene primer set were: (27F: 5' - AGA GTT TGA TCC TGG CTC AG - 3' and

1492R: 5' -CCG TCA ATT CCT TTG AGT TT- 3') was used to accomplish PCR amplification (Edwards et al., 1989).

PCR reaction mixture contained 50 µl of master mix (1.25 U Hot Start Taq DNA Polymerase (Solis Biodyne, Estonia), 25-50ng/µl of DNA template, 0.3 µMµl of each primer1.5 ,µM MgCl2 Magnesium chloride (Solis Biodyne ,Estonia), adding distilled water, and increased the reaction volume to 50 µl. The PCR procedure involved the following steps:

- Initial denaturation: was placed at 94°C for 12 minutes;
- Second denaturation: at 94°C for 1 minute;
- Annealing at 55°C for 1 minute;
- Extensions at 72°C for 1 minute.

The amplification processes was repeated 30 times followed by a final extension at 72°C for 7 minutes. PCR was conducted using a thermocycler (iCycler Bio-Rad, USA). Moreover, the DNA concentrations were analysed with a Nano drop Spectrophotometer (NanoDropTM 2000 ,USA). The PCR obtained product was separated on a 1.5% agarose gel (Sigma-Aldrich, USA). Following the PCR process as DNA molecular weight markers, a100 base pair (bp) DNA ladder (Solis Biodyne, Estonia) was employed. Subsequently, 90 minutes of electrophoresis at 80 V and gel viewing under UV light following staining with Midori Green Advance (Nippon Genetics, Japan) and inspected with a UV trans-illuminator.

The PCR obtained products were electrophoresed, and purified by the use of a Vivantis Clean Up kit, then sent to a sequencing company (Apical Scientific Sdn. Bhd.). BLASTn that is available on the NCBI website (http://blast.ncbi.nlm.nih.gov) was used to analyse the produced sequences and sequencing evaluation. In independent processes and duplicates, forward and reverse sequencing of purified PCR results was performed. The ingredients for each reaction were 40 µg of template DNA, 2µ l of the proper PCR primer, 10µ l of water ,and µ2 l of the Big Dye Terminator v3.1 Ready Re-action Mix (Applied Biosystems). For 25 cycles ,each reaction was heated to 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 s. This process took each reaction one minute to reach 96 °C. To eliminate unincorporated reagents and guarantee a neutral charge, the sequencing products were purified using the ethanol precipitation technique .Briefly, the DNA was pelleted by centrifugation after the sequencing products were washed in 80 µl of ethanol precipitation mix (3µ l NaAc, 62.5 µl 95% ethanol, and 14.5 µl water) (13 15 ,000 min). The particle was centrifuged after being cleaned one again in 200µ l 75% ethanol (13 5 ,000 min). The pelleted DNA was placed onto a 3130 Genetic Analyzer Capillary Array for detection after being air dried and rehydrated in 15µ l formamide (Applied Biosystems). Using Bionumerics v3.5 (Applied Maths), two forward and two reverse sequences for each sample were aligned to produce a composite sequence. Each sequence trace's

quality was evaluated visually, and the low-quality sequences were modified, and eliminated. The creatures described in each article were located by comparing consensus sequences to a database (Belgacem et al., 2023).

b) 16s rDNA gene and phylogenetic analyses:

The identification of organisms were realised by comparing consensus sequences, the phylogenetic neighbors was initially carried out by using the Basic Local Alignment Search Tool **BLAST** platform from NCBI Data base (https://blast.ncbi.nlm.nih.gov/). The sequence obtained was submitted a web based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains . The phylogenetic relationship between the isolate and closely related species was investigated using Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis **MEGA7** program

3.3.4. Extraction of the crude extract:

By the use of GYE medium, after 7 days of incubation at 31°C of the selected Actinobacteria, the bioactive compounds were extracted via maceration method using acetate Ethyl. Indeed, the Actinobacteria on plate agar were sliced into $1cm^2$ fragments before being placed in a bottle containing 50 ml of ethyl acetate. The maceration mixtures were under constant agitation over 48 hours at room temperature. Subsequently, separating the solvent for agar blocks and mycelium with Whatman paper **N°1**, the crude extracts were obtained. The liquid were evaporated to dryness using rotary evaporator equipment. The resultant dry extracts were recovered in 5 ml of methanol and stored at 4°C until farther use (Boughachiche et al., 2012).

3.3.5. Actinobacteria extract and anti-biofilm essays:

Based on the results of preliminary screening the Actinobacteria that have showed an interesting antagonistic activity were directed to evaluate their anti-biofilm activity concurrently with qualitative biofilm detection as described above (section Quantitative analysis of biofilm production). All the tested clinical strains were grown in 96-well polystyrene plates in the presence and absence of Actinobacteria extracts at 37°C/24h. The chosen volume of the inhibitory quantity was 20µl for each methanolic extract as realized by (Saleem et al., 2015).

The anti-biofilm activity was categorized as inhibition or elimination. To detect inhibition activity, pathogens (0.5 Mc) cultivated into Brain Heart Infusion Broth, and extracts were transferred to the 96-well microplate. Biofilm inhibition activity was quantified after 24 h. For the elimination activity assay, another 96-well plate with bacterial culture was incubated at 37 °C for 24 h. After biofilms were attached, extract was added and incubated for 24 h. Each pathogenic culture was used as the

positive control, while sterile BHIB was used as the negative control. The manipulation was repeated for three times.

After incubation, planktonic cells and media were discarded. Adherent cells were rinsed with sterile water and stained with crystal violet (0.5% w/v) for 30 min. The microplate was rinsed and air-dried for 5 min. Subsequently, 200 µL of ethanol was added for CV dye elution. Absorbance was determined at λ max 630 nm in an enzyme-linked immunosorbent assay reader (ELISA) plate reader (Bio-Tek Instruments, USA) (Harika et al., 2020)

***N.B*: all the related statistical analyses were realized by the use of Microsoft office Excel 2016 and Xl STAT programs trial version 2024.**

CHAPTER THREE: *RESULTS AND DISCUSSION*

1. Clinical strain and biofilm detection essays:

1.1.Assessment of Clinical photogenic strains:

In the present study, from the thirty clinical samples, 20 clinical isolates varied between *S aureus, P aeruginosa* and *E coli* were obtained (**tab. 3, tab.4).** The majority of the clinical isolate were detected in pus and urine samples respectively.

Table 3: Frequency and distribution of the isolated clinical strains

Bacterial isolates	Total No. (%)	Gender	Sample type			
			Urine	Catheter urine	pus	Chirurgical sites
			No. (%)			
S aureus	9. (45)	M	0. (0)	0. (0)	3. (33.33)	0. (0)
		F	0. (0)	0. (0)	6. (66.66)	0.00
P aeruginosa	2 (10)	M	0. (0)	0. (0)	1. (50)	0. (0)
		F	0. (0)	0. (0)	1. (50)	0. (0)
E coli	9 (45)	M	4. (44.44)	0	0. (0)	0. (0)
		F	5.(55.55)	0	0. (0)	0. (0)

Table 4: Result of MALDI-TOF clinical strains identification

Sample Name	Sample ID	Organisme (best match)	Score Value	Organisme (second-best match)	Score value
A3/A4 (+++)(A)	SaPF01	*Staphylococcus aureus*	2.31/2.39	*Staphylococcus aureus*	2.10/2.21
A5/A6 (+++)(A)	SaPM02	*Staphylococcus aureus*	2.38/2.49	*Staphylococcus aureus*	2.33/2.43
A7/A8 (+++)(A)	SaPF03	*Staphylococcus aureus*	2.35/2.17	*Staphylococcus aureus*	2.35/2.16
A9/A10 (+++)(A)	SaPM04	*Staphylococcus aureus*	2.23/2.38	*Staphylococcus aureus*	2.10/2.30
A11/A12 (+++)(A)	SaPF05	*Staphylococcus aureus*	2.38/2.19	*Staphylococcus aureus*	2.26/2.14
B3/B4 (+++)(A)	SaPF07	*Staphylococcus aureus*	2.45/2.44	*Staphylococcus aureus*	2.24/2.18
B5/B6 (+++)(A)	SaPM08	*Staphylococcus aureus*	2.46/2.50	*Staphylococcus aureus*	2.12/2.17
B7/B8 (+++)(A)	SaBCPF 09	*Staphylococcus aureus*	2.40/2.40	*Staphylococcus aureus*	2.16/2.16
B9/B10 (+++)(A)	SaPF10	*Staphylococcus aureus*	2.44/2.44	*Staphylococcus aureus*	2.17/2.30
C1/C2 (+++)(A)	PaPM01	*Pseudomonas aeruginosa*	2.18/2.28	*Pseudomonas aeruginosa*	2.16/2.27
D3/D4 (+++)(A)	PaPM09	*Pseudomonas aeruginosa*	2.48/2.41	*Pseudomonas aeruginosa*	2.33/2.31
D7/D8 (+++)(A)	ECUF01	*Escherichia coli*	2.40/2.46	*Escherichia coli*	2.26/2.40
D9/D10 (+++)(A)	ECUF02	*Escherichia coli*	2.29/2.31	*Escherichia coli*	2.20/2.30
D11/D12 (+++)(A)	ECUF03	*Escherichia coli*	2.34/2.45	*Escherichia coli*	2.32/2.43
E1/E2 (+++)(A)	ECUM04	*Escherichia coli*	2.47/2.35	*Escherichia coli*	2.40/2.08
E5/E6 (+++)(A)	ECUM06	*Escherichia coli*	2.39/2.39	*Escherichia coli*	2.34/2.38
E7/E8 (+++)(A)	ECUM07	*Escherichia coli*	2.39/2.38	*Escherichia coli*	2.37/2.37

E9/E10 (+++)(A)	ECUF08	*Escherichia coli*	2.36/2.38	*Escherichia coli*	2.26/2.36
E11/E12 (+++)(A)	ECUM09	*Escherichia coli*	2.34/2.38	*Escherichia coli*	2.22/2.37
F1/F2 (+++)(A)	ECUF10	*Escherichia coli*	2.19/2.32	*Escherichia coli*	1.99/2.30

1.2.Evaluation of multidrug resistance and β-lactamase production:

Among the total isolates (n= 20), the overall prevalence multidrug were detected in 11 strains of all clinical bacteria. In this study, the MDR recorded in both of Gram positive and Gram Negative bacteria.

Penicillin inhibits the bacterial peptidoglycan synthesis, which is the major cell wall component. It exhibits rigid mechanical stability due to its highly cross linked lattice wall structure in the bacterium (Pugazhendhi et al., 2020). In contrast, in this study all the *S. aureus* strains (100 %) were highly resistant to Penicillin G, this resistance due to the β-lactamase enzyme production .Whereas, inherent weakness of penicillin is because of the attack of ring nucleus by β-lactamase produced in *S. aureus* that rendered penicillin inactive. These strains considered as β-lactamase producing Staphylococcus (BLACT). In addition, from the nine BLACT staphylococci two of them are Methicillin Resistance Staphylococcus (MRSA) strain *S. aureus* 01 and 02, their resistant exceed to cefoxitine and oxacilline (>2µg/ml) as methicillin resistance marker. The isolated MRSA are also resistant to vancomycin, which is usually the first line antibiotic for MRSA related infections (Paleczny et al., 2022).

Furthermore, from the 9 isolates *Escherichia coli* one of them *E. coli 09* was recorded as MDR-*E.coli* with Extended Spectrum Beta-lactamase (ESBL), whereas *E. coli* 04 considered as class D Carbapenemase Producers (CARBD).

ESBL E.coli showed a elevate resistance to cephalosporin antibiotic groups Cefazolin (>32), Cefepime (16), Cefixim (>2), Cefotaxime (>4), Ceftazidime (>16), Ceftriaxone (>4), Cefuroxime (>16), also for ciprofloxacin and levofloxacin, Gentamycin. On the other hand, ESBL E.coli susceptible to Erthapenem (≤0.25) and Meropeneme (≤0.125) as Carbapenems antibiotic class. (Bush & Bradford, 2020) have been clarified that ESBL phenotype in that they confer resistance to some of the late-generation Cephalosporins. Many of these are derived from OXA-10 and OXA-2 and are commonly found in *P. aeruginosa*. OXA-163 is an interesting variant of OXA-48 in that it has an ESBL phenotype but is not a Carbapenemase. Extended spectrum β-lactamase producing pathogenic cause a serious antibiotic management problem, as the enzyme encoding genes are easily transferred between organisms via conjugation way (Sahle et al., 2022).

While, CARBD *E.coli* showed a resistance to Amoxicillin (>32) , Amoxiciline-Clavulanate (>32/2) Ampicillin (>16), Ciprofloxacin, Levofloxacin, an intermediate susceptibility to Ertapenem (=1) and susceptible to Imipenem (=0.5). (Antunes et al., 2014) have been reported a similar results, which observed in our results that the expression of carbapenemas in the *E. coli* background produces only

low levels of resistance to carbapenems. In contrast, the results demonstrated that the isolated CARBD *E.coli* susceptible to cephalosporins and only modestly elevated Carbapenem MIC values, with many Imipenem MICs of ≤2 µg/ml, this isolate could be an OXA-48 carbapemenase as described by (Bush & Bradford, 2020).

On the other hand, Gram-negative bacteria such as *Pseudomonas aeruginosa* showed a large spectrum of cephalosporins resistance. *P. aeruginosa* resistant to more than eleven antibacterial agents as Amoxicillin (>32) , Amoxicillin Clavulanate (>32/2) , Ampicillin(>16), Ampicillin-Sulbactam (>4/8), Cefazolin (>32), Cefotaxime (>4), Ceftazidime (>16), Ceftriaxone (>4), Cefuroxime (>16), Colistin (>4) and Ertapenem (>2). However, they considered as Non MDR-*P. aeruginosa* (**Annex. 4**)

1.3. Adherence assays and evaluation of biofilm production ability:

1.3.1. Phenotypic characterization of slime synthesizing strains using CRA and TAM.

The results of CRA method demonstrated that the strong biofilm productivity represented by 10% *E.coli* 09, they appeared with black colonies. The moderate biofilm production represented by 40% of all the strains, 15% *S. aureus* (SA: 01, 04 and 05), with 15% *E.coli* (EC: 03, 07 and 08), followed by *P. aeruginosa* 10% (PA: 01and 09), they showed colonies with a glossy crystalline dark gray to brown color. The weakly productive strains was 50% of the clinical strains presented by 30% *S. aureus* (SA 02, 03, 07, 08 and), followed by 20% of *E. coli* (EC 01, 02, 04 and 06) which appeared with red to pink colonies. On the other side the result of TAM related with the degree of pigmentation with crystal violet are completely separated from the results of CRA method. While the E. coli strains are the stronger producers 100%, and *P. aeruginosa* 100%. The *S. aureus* strains are 100% weak biofilm producers. The results showed in the (**fig. 16, tab. 5**).

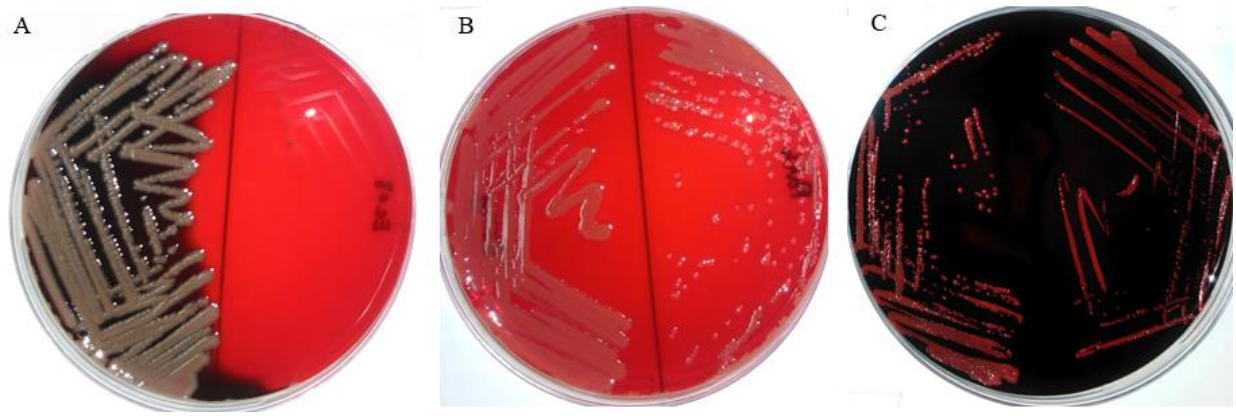


Figure 16: Congo Red Agar assay

A: *E. coli* with gray colony; B: *P. aeruginosa* with brown colony; C: *S. aureus* with red colony

Table 5: Results of phenotypic biofilm detection using CRO and TAM method

Clinical Strains	Total N	Biofilm production (%)					
		TM			CRO		
		Strong	Moderate	Weak	Strong (black - Gray CFU)	Moderate (brown or gay CFU)	Weak (Red CFU)
S. aureus	9	0 (0%)	0 (0%)	9 (100%)	0 (0%)	3 (15%)	6 (30%)
E. coli	9	9 (100%)	0 (0%)	0 (0%)	2 (10%)	3 (15%)	4 (20%)
P. aeruginosa	2	10% (2)	0 (0%)	0 (0%)	0 (0%)	2 (10%)	0 (0%)

From the results of the current study, we note that there is a discrepancy in the ability to produce biofilms by Gram-positive and Gram-negative bacteria using the Congo red agar and in TAM methods. This difference may be due to the discrepancy in the sensitivity of both methods, and this has been confirmed by many studies. The researcher (Obaid, 2019) indicated that 35% of *S. aureus* are biofilm producers, while 47% of *E. coli* are biofilm producers by using the Congo red agar method, these results are incompatible with what we found. The results of the used methods to detect biofilm production are different, and they mainly include the CRA and TAM method. Although there is no relationship between the two methods, they are the easiest to qualitatively detected biofilms; however, they are the perfect in quantitative estimation of the biofilm production.

In the other side, the CRO method is a virtual method for distinguishing the phenotypic pattern of biofilm-forming bacteria. ether are high, medium or low virulence, which will reflect the severity of the infection and this will help in the determination of the initial treatment, as it depends on enhancing the production of exopolysaccharide using a rich medium such as BHI medium (Mathur et al., 2006). Congo red is a diazo textile dye that has been used for over a century to visualize the development of amyloid fibers. Later, microbiological uses emerged, particularly in detecting bacteria that form amyloid appendages known as curli and overexpressing polysaccharides in the biofilm matrix. The second messenger cyclic diguanylate (c-di-GMP) regulates the production of biofilm matrix polysaccharides, and therefore Congo red staining of samples can be utilized as an indirect measurement of elevated c-di-GMP production in bacteria. Congo red enable the identification of strains producing high c-di-GMP in an inexpensive, quantitative, and high-throughput method (C. J. Jones & Wozniak, 2017).

1.3.2. In vitro adherence assay on polystyrene microtitre plate (MTP)

When using the MTP method to estimated the biofilm formation ability. The method showed completely different results, the results demonstrated that the majority of the isolated clinical bacteria

were appeared to be moderate biofilm productive (95 %), whereas (5%) as weakly biofilm productive, and (0%) as no productive biofilm bacteria. In fact, these results were considered the clinical bacteria as 100% biofilm producers. Thus, the strains *S. aureus* OD_{600} ranging between [0.33- 0.75], While *E.coli* strains OD_{600} ranging in [0.50- 0.74] and for *P. aeruginosa strains* [0.60- 0.66].

Furthermore, the standard CV staining method MTP, applied in a 96-well microplate, has made it possible to demonstrate the capacity of all isolated strains to form biofilms after 24 hours of incubation with constant intensities of CV staining 0.02%. Indeed, strain *S. aureus* (SA: 01, 02 and *09), E coli (EC:* 06 and 08 were the highest productive with OD_{600}= 0.75, 0.74, 0.73 and 0.74, 0.74 respectively, the other strains are in average of OD_{600}= 0.49 to 0.69, while the *S. aureus* strain SA 05 was weakly biofilm-forming with OD_{600}=0.33. The results are cleared in **fig. 17**

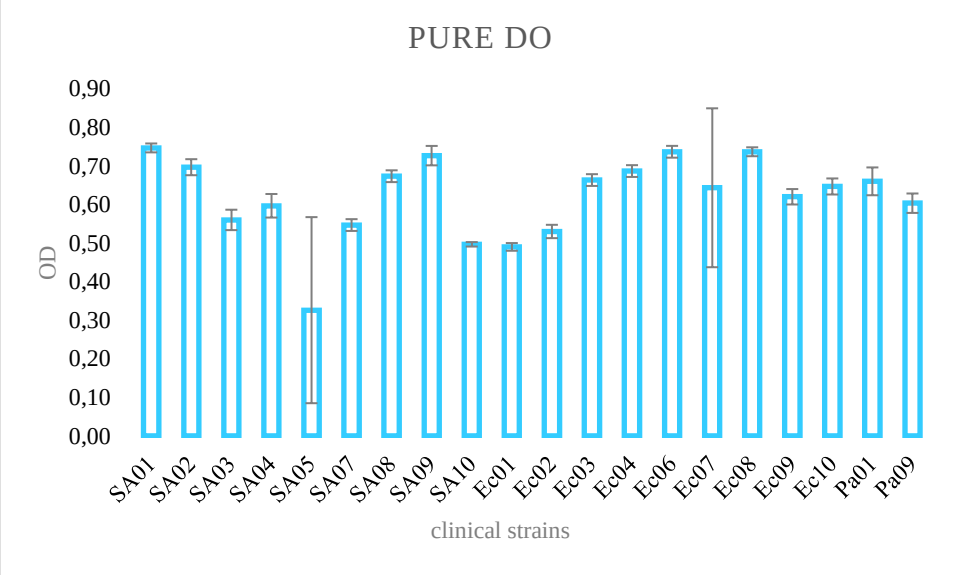


Figure 17: MTP results of biofilm producing clinical bacteria

The results showed that the MTP method was more sensitive in detecting biofilm formation compared to Congo Red Agar and the tube adherent method, while this method gave the highest positive percentage for biofilm production compared to Congo Red assays, which had a positive production percentage (95%). The results also showed that the weakly productive isolates were (5%) by the MTP method, while their percentage was (50%) by the Red Congo method. As for the percentage of non-productive isolates by the MTP method, it was (0%) while their percentage was also (0%) by the Red Congo method. We also note that the total percentage of biofilm production in its strong forms reached by the MTP method. The current results agreed with the researcher's results as the bacteria producing biofilm were moderately (Obaid, 2019), while the bacteria producing strongly were few and there were low of non-biofilm producing bacteria at a rate of. Which did not agree with our result From the results of the current study (Picoli et al., 2017), we note that there is a difference in the ability to form biofilm between Gram-negative and Gram-positive bacteria using the MTP method,

Congo red, and tube adherent methods. This difference may be due to the sensitivity of each of the mentioned methods. This is what many studies have confirmed (Knobloch et al., 2002).

The formation of the biofilm varies according to the bacterial strain, the growth medium, and the degree of hydration (Zhao et al., 2023; Zou & Liu, 2018). The Investigation of biofilm formation also depends on the detection method and incubation conditions, as well as the type of abiotic surfaces, whether they are growth on glass, polystyrene, silicone, etc. Studies confirm that polystyrene is the most affected by Biofilms.

The results of biofilm formation using the MTP method vary depending on the strain studied and the medium used for growth. After studying the early stages of biofilm formation, we can use the MTP method because this method uses a fixed environment, so it can be used to study many of the necessary factors involved in the biofilm formation process, such as flagella, hyphae, enzymes, etc.

2. Soil *Actinobacteria* sp., as promising agents of biofilm biological control:

2.1.Colony and bacterial morphological traits:

The study was performed to isolate Actinobacteria strains with antibacterial activity from Acacia Senegal rhizosphere soil from Tamanrasset region located in southern Algeria. I fact, after five days 124 different strains were isolated on GYE media from seven soils samples of Tamanrasset town. GYE media seems to be selective for Actinobacteria, because it contain Glycerol that most of Actinobacteria use it as a carbon source (Abussaud et al., 2013; Oskay et al., 2004; Reggani et al., 2021). The Glycerol and Saharan soil mediums appear to play to main roles as modulators and regulators in biosynthesis of inhibitory substances, in some Actinobacteria sp that we have isolated from Tamanrasset rhizosphere soil, it well now that the Glycerol utilization involved in Clavulanic acid, with a potent of β-lactamas inhibitor produced by *Streptomyces Clavuligerus* (Fu et al., 2019).

Besides, all the seven soil samples have a great productivity of Actinobacteria isolates, the obtained colonies exhibit on GYE-agar media the typically Actinobacteria phenotypes with pinpoint , powdery, chalky and dry colonies, whose diameters vary from 01 to 09mm. Such characteristics are already reported by previous several studies (Barka et al., 2016a; Zacchetti et al., 2018). All colony exhibit an aerial and substrate mycelium with different colors viz: white, beige, orange ,light orange and dark green, with mostly, dominance of the white color. In fact, 100% of Actinobacteria isolates colony white aerial mycelium and substrate mycelium. These criterions are well established by the various bibliographic data. On the other hand, all isolates under light microscopic observation are Gram positive and have a similar filamentous morphology typical to Actinomycetes sp as described by previous study, which exhibit a branched substrate mycelium with presence of aerial hyphae (Dilip et al., 2013; Lawson, 2018). **(tab. 6, Annex. 03)**.

Table 6: Macroscopic characterization of the Isolated Actinobacteria

Strain code	Frequency	Consistency	Aerial l mass color	Reverse side pigment	Soluble pigment	Diameter
AB-B1-4 str1	1	Circular, plate, regular edge, smooth	Vivid yellow	Vivid yellow	no	9
AB-B1-4 str4	1	Circular, plate, regular edge, smooth	pale yellowish pink	pale yellowish pink	no	2
AB-B1-4 str5	1	Circular, plate, regular edge, smooth, convex	strong reddish orange	strong reddish orange	no	1
AB-B1-4 str6	1	Ovular, perforated; regular edge, smooth, convex	pal greenish yellow	pal greenish yellow	no	4
AB-B1-4 str7 **D39**	2	Circular, convex ,regular edge, smooth	moderate reddish orange	moderate reddish orange	no	0,5
AB-B1-4 str8 **D38**	1	Circular, convex, irregular edge, rough	grayish olive	grayish olive	pale yellow	1
AB-B1-4 str9	1	Circular, convex with pointed center, irregular edge, rough	strong reddish orange	strong reddish orange	moderate reddish orange	2
AB-B1-4 str10	1	Circular, plat regular edge, smooth	pale yellowish pink	pale yellowish pink	no	2
AB-B3-4 str13 **D1**	1	Volcanic shape, circular, regular edge, perforated with cotton texture	pale yellowish pink	light yellowish pink	no	4
AB-B1-4 str17	1	Circular, plat regular edge, smooth	pale yellowish pink	pale yellowish pink	no	2
AB-B1-4 str18	1	Circular; convex , with higher center regular edge, smooth,	brilliant yellow	brilliant yellow	no	8
TI-B1-4 str19	1	Circular, plat regular edge, smooth	white	strong orange yellow	deep reddish orange	5
TI-B1-4 str20	4	Flower shape, plate, irregular edge, rough	deep greenish	deep orange yellow	no	6
TI-B1-4 str21	3	Circular striped, plat with pointed center, regular edge, smooth	beige	beige	no	7
TI-B1-4 str22 **D36**	2	Circular, convex, regular edge, rough	pale yellow green	grayish olive	light yellow	6
TI-B1-4 str23 **D4**	1	Volcanic shape, plat ,regular edge, with cotton texture	beige	light yellow	no	6
TI-B1-4 str24	1	Circular stripped, convex with pointed center, regular edge, rough	beige Wight orange yellow	beige	no	5
TI-B1-4 str25	2	Circular, convex, regular edge, smooth	vivid greenish yellow with brilliant yellow center	vivid greenish yellow	no	4
TI-B1-4 str26 **D40**	3	Circular, plat regular edge, rough	beige	beige	no	6
TI-B1-4 str27 **D35**	3	Circular, plat, , perforated, irregular edge, rough,	beige	beige	no	4
TI-B1-4 str28 **D21**	3	Circular, convex, irregular edge, rough,	beige	beige	no	4
TI-B1-4 str29	1	Circular, plat, perforated, regular edge, smooth	brilliant greenish yellow	brilliant greenish yellow	no	3
TI-B2-4 str30	2	Circular volcanic shape, plat ,irregular edge, with cotton texture	white	beige	no	9
TI-B2-4 str31	3	Circular, plat, triangle perforated, regular edge, smooth	moderate yellow brown	moderate yellow brown	Pal violet	6
TI-B2-4 str32	1	Flower shape, plat ,irregular edge, rough	brilliant beige	beige	no	4

TI-B2-4 str33	1	Circular, plat, perforated, regular edge, smooth	light orang yellow	light orang yellow	no	5
TI-B2-4 str34	1	Circular, convex, regular edge, smooth,	dark orange yellow with beige center	dark orange yellow with beige center	moderate orange	5
TI-B2-4 str35	2	Circular, plat, regular edge, smooth,	grayish yellowish brown	grayish yellowish brown	no	2
TI-B2-4 str36	1	Circular, convex, irregular edge, rough,	white	beige	no	4
TI-B2-4 str37	3	Circular, plat, regular edge, smooth,	beige	beige	no	5
TI-B2-4 str38	1	Circular stripped (lemon shape), regular edge, smooth	beige	beige	no	7
TI-B2-4 str39	3	Irregular shape, stripped, convex, irregular edge, rough, corrugated.	white	red	dark red	1
TI-B3-4 str40 **D18**	1	Circular flower shape , convex, irregular edge, rough,	pale yellow green	greenish olive	pale yellow	6
TI-B3-4 str41 **D33**	1	Irregular shape, cracked, convex, irregular edge, rough,	light yellow	light yellow	on	6
TI-B3-4 str42	1	Circular, convex, regular edge, smooth	brilliant beige	brilliant beige	on	3
TI-B3-4 str43	5	Circular, convex, regular edge, smooth	beige	beige	on	3
TI-B3-4 str44 **D19**	1	Circular, convex, irregular edge, rough	beige	beige	on	4
TI-B3-4 str45	1	Circular, convex, irregular edge, rough	strong yellow pink	strong yellow pink	light yellow pink	4
TI-B1-5 str46 **D24**	1	Circular, plat, irregular edge, smooth	beige brilliant orang center	beige	no	4
TI-B1-5 str47 **D32**	1	Circular, plat, regular edge, smooth	beige	beige	no	5
TI-B1-5 str48	1	Circular stripped (lemon shape), regular edge, smooth	beige	beige	no	7
TI-B1-5 str49	1	Circular, plat, perforated, regular edge, smooth	beige	beige	no	6
TI-B1-5 str50	1	Circular, plat, irregular edge, rough	white	beige	no	6
TI-B1-5 str51 **D31**	1	Circular, convex, regular edge, smooth	light yellow with brilliant orange center	light yellow	no	4
TI-B1-5 str52 **D42**	1	Circular, plat, regular edge, smooth	beige	beige	no	1
TI-B2-5 str53	1	Circular, convex, stripped regular edge, smooth	light yellow	light yellow	no	2
TI-B3-5 str54 **D30**	1	Circular, plat, regular edge, smooth	light yellow	light yellow	no	7
TI-B3-5 str55 **D48**	1	Circular, plat, , regular edge, wrinkled	white	white	no	10
TI-B3-5 str56	1	Circular, plat, perforated, regular edge, wrinkled	light yellow	light yellow	no	6
TI-B3-5 str57	1	Circular, plat, irregular edge, rough	light yellow	light yellow	no	7
TI-B3-5 str58	1	Circular, plat, , regular edge, smooth	brilliant yellow	brilliant yellow	no	5
TI-B3-5 str59	2	Circular, plat, , regular edge, smooth	beige	beige	no	4
TI-B3-5 str60	1	Circular, convex, perforated, irregular edge, rough	white	Moderate yellowish green	no	6

OT-B1-4 str61	2	Circular, plat, , regular edge, cotton texture	vivid yellow	vivid yellow	no	5
OT-B1-4 str62	4	Circular, plat, perforated, regular edge, wrinkled	beige	beige	no	4
OT-B1-4 str63	1	Circular, convex, perforated , regular edge, rough	white	beige	moderate orange yellow	5
OT-B1-4 str64 **D11**	1	Circular, plat, , irregular edge, rough	beige	beige	moderate orange yellow	3
OT-B2-4 str65	1	Circular, plat, , irregular edge, rough	light yellow	light yellow	no	6
OT-B2-4 str66	1	Circular, plat, , irregular edge, rough	beige	beige	no	6
OT-B2-4 str67	1	Circular, convex, regular edge, smooth	Wight	pinkish gray	no	5
OT-B2-4 str68	1	Brane shape, convex, irregular edge, wrinkled	Wight	pinkish gray	no	6
OT-B2-4 str70	1	Circular, convex, perforated , regular edge, rough	brilliant yellow	brilliant yellow	no	2
OT-B2-4 str71	1	Circular, plat, , regular edge, rough	beige	beige	no	7
OT-B2-4 str72	1	Circular, convex, ,perforated, regular edge, smooth	pinkish gray	pinkish gray	no	3
OT-B2-4 str73	1	Circular, plat, , regular edge, smooth	orang	orang	no	2
OT-B2-4 str74	2	Circular, convex, , regular edge, smooth	beige	beige	no	2
OT-B2-4 str75	1	Circular, plat, perforated, irregular edge, rough	light yellow	light yellow	no	4
OT-B2-4 str76	1	Circular, convex, , irregular edge, wrinkled	strong yellow	strong yellow	no	7
OT-B2-4 str77	1	Circular, convex, , irregular edge, rough	light yellow	light yellow	no	7
OT-B1-5 str79 **D12**	1	Circular, convex, regular edge, wrinkled	vivid yellowish pink	vivid yellowish pink	no	1
OT-B1-5 str80	1	Circular, convex, regular edge, wrinkled	strong yellow	strong yellow	no	1
OT-B1-5 str81	1	Circular, convex, regular edge, smooth	beige	beige	no	3
OT-B1-5 str82	1	Circular, convex, perforated, irregular edge, wrinkled	strong yellowish brown	strong yellowish brown	no	2
OT-B1-5 str83	1	Circular, plat, stripped (lemon shape), regular edge, wrinkled	beige	beige	no	10
OT-B1-5 str84 **D27**	1	Circular, plat, stripped, perforated, regular edge, wrinkled	white	beige	no	9
OT-B1-5 str85	1	Circular, convex, regular edge, smooth	beige	beige	no	5
OT-B1-5 str87	1	Circular, plat, regular edge, wrinkled	light yellow	light yellow	no	6
OT-B1-5 str88	1	Irregular shape, irregular edge, rough	beige	beige	no	6
OT-B1-5 str89 **D45**	1	Circular, convex, regular edge, smooth	moderate orange yellow	moderate orange yellow	no	3
OT-B1-5 str90 **D22**	1	Circular, convex, regular edge, smooth	greenish Wight	dark olive brown	dark olive brown	1
AG-B2-4str95	1	Circular, convex, irregular edge, wrinkled	white, gray center	white	no	5

AH-B1-4str96	2	Circular, convex, irregular edge, cotton texture	white	white	grayish pink	5
AH-B1-4str97	2	Circular, convex, regular edge, cotton texture	very pale green	very pale green	pal green	4
AH-B2-4str98	1	Circular, convex, regular edge, cotton texture	very pale green	very pale green	no	7
IN-B1-4str99	1	Circular, convex, regular edge, cotton texture	dark gray Wight	dark gray	no	4
IN-B1-4str100 **D14**	5	Circular, convex, regular edge, smooth	white	dark gray pink	no	4
AD-B1-4 str101	4	Circular, convex, regular edge, cotton texture	white	white	no	2
AD-B2-5str102 **D15**	1	Circular, plat, center pointed regular edge, wrinkled	white, light yellow center	light yellow	strong yellow	7
AD-B2-5str103	1	Circular, convex, regular edge, smooth	beige	beige	no	3
AD-B2-5str104 **D16**	1	Circular, plat, stripped (lemon shape), regular edge, smooth	white	beige	dark yellowish pink	10
AD-B2-5str105	1	Circular, plat, regular edge, wrinkled	beige	beige	no	5
AD-B3-5str106	1	Circular, plat, irregular edge, rough	white	gray	dark yellowish pink	6
AD-B3-5str107	1	Circular, plat, irregular edge, rough	grayish olive	grayish olive	no	5
AD-B3-5str109	1	Circular, plat, pointed center irregular edge, rough	light orang	light orang	no	3
AD-B3-5str110	2	Circular, plat, pointed center irregular edge, rough	beige	beige	no	6
TI-B1-4- str112	1	Circular, plat, regular edge, cotton texture	Light brownish gray	Strong brown	no	5
TI-B1-4- str114	1	Circular, plat, irregular edge, wrinkled	Dark grayish brown	Dark grayish brown	Deep brown	4
TI-B2-4- str115 **D47**	1	Circular, plat, stripped, regular edge, smooth	beige	beige	no	4
TI-B2-4- str116	1	Circular, plat, regular edge, wrinkled	White	beige	no	6
TI-2-4- str117	1	Circular, convex, regular edge, smooth	beige	beige	no	1
IN-B1-4- str119	1	Circular, convex, regular edge, smooth	Light yellow	Light yellow	no	7
IN-B1-4- str121 **D26**	1	Circular flower shape, convex, irregular edge, rough	White	beige	no	6
IN-B1-4- str122	1	Circular, convex, regular edge, smooth	Light pink	White	no	1
AB-B1-6str124 **D25**	1	Circular, plat, regular edge, cotton texture	Light yellowish brown	Dark orang yellow	Dark orang yellow	3

Some Actinobacteria species have been displayed to be significant in the rhizosphere, where they defence roots from harmful infection diseases and may boost plant growth. They have the ability to create active molecules, such as antifungal and antibacterial compounds, siderophores, or plant growth regulators. Some Actinobacteria have also been linked to plant growth by forming symbiotic relationships with crop plants and colonizing their internal tissues without creating disease symptoms. This led us to thinking about their impact on the health and medical filed (Zamoum et al., 2015).

2.2.Screening of Antibacterial activity of the isolates

Out of the 46 Actinobacterial cultures screened for antibacterial activity, 20 cultures were found to produce active products against various pathogenic microorganisms such as Gram-negative and Gram-positive bacteria ,using cross streak method, an example was showed in **Figure 18:**

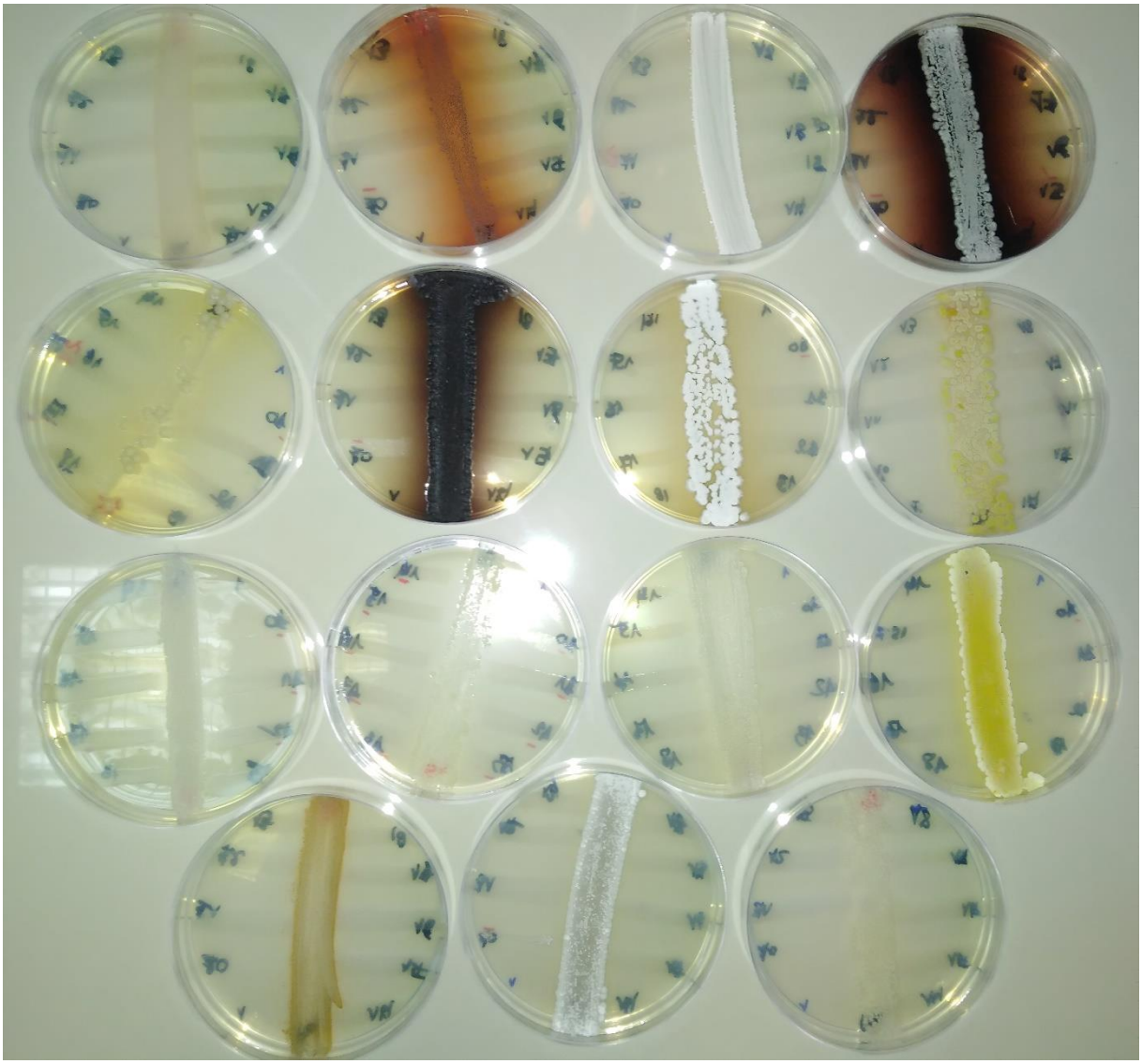


Figure 18: Cross streak assay and the antagonistic Activity of some Actinobacteria against P. aeruginosa clinical strains

The antagonistic activity of the isolated Actinobacteria varied between no antagonistic activity, selective antagonistic activity and large antagonistic activity. as recorded in many studies (Al-Ansari

et al., 2020; Elbendary et al., 2018; Rammali et al., 2024), the majority of the isolated Actinobacteria are highly active against all *S. aureus* strains as Gram-positive bacteria, with inhibition zone ≤ 35 mm. The antagonistic activity were decreased in the interaction with E. coli, only six Actinobacteria have the antibacterial activity at most of clinical *E. coli* strains with inhibition zone ≤ 25 mm.

The Actinobacteria strains D25, D32 and D48 showed a selective activity against all *S. aureus* strains with average of inhibition zone (mm) varied [4-20], [2-8] and ≤ 4 respectively. While the Actinobacteria strains D24 and D42, have a selective activity only against *E. coli* strains (all tested strains) with inhibition zone [6-20], [5-11] respectively.

On the other side, The Actinobacteria strains D31, D33, D35, D36 and D47 have been registered the largest antagonistic activity [3-6], [2-5], [20-35], [20-34] and [7-15] respectively against all *S. aureus* strains, as well as against all *E.coli* strains [5-11], [2-7], [18-25], [4-6] and[4-20] respectively. It should be noted that the strain D35 are the best isolate on their antagonistic activity. However, no antagonistic activity have recorded by the strains D1, D11, D12, D14, D21, D22, D26, D27, D28, D30, D32, D38, D39, D40 and D45. Furthermore *P. aeruginosa* are more resisted to Actinobacterial bioactive compound, these results are frequently detected in the academic research studies (Meklat et al., 2020; Mohamed et al., 2017; R. Singh & Dubey, 2020).

2.3. Effect of the crude extract against biofilm formation and quantification of anti-biofilm activity

Frome the results of MTP assays, the strains were noted as biofilm forming clinical strains, their ability to adhere varied from weakly to moderate biofilm producers (OD_{630nm} ranging between [0.33-0.75]). The addition of Actinobacteria crude extract with 20µl to the growth medium achieve a significant results against *S. aureus, E. coli* and *P. aeruginosa* biofilms as presented in (**fig. 19, tab. 7**).

Table 7: Effect of the crude extracts against S. aureus biofilm formation

Modality	SA01	SA02	SA03	SA04	SA05	SA07	SA08	SA09	SA10
EX19	0,747 a	0,697 a	0,560 b	0,597 a	0,326 a	0,547 a	0,673 a	0,727 a	0,497 ab
EX104	0,607 b	0,563 bc	0,493 bc	0,600 a	0,370 a	0,523 a	0,583 b	0,683 a	0,497 ab
EX40	0,560 bc	0,570 bc	0,347 cd	0,513 bc	0,467 a	0,457 bc	0,613 ab	0,610 a	0,537 ab
EX44	0,560 bc	0,537 bc	0,467 bc	0,525 b	0,433 a	0,475 b	0,648 a	0,485 a	0,452 b
EX102	0,613 b	0,573 b	0,313 d	0,540 b	0,317 a	0,463 b	0,630 ab	0,467 a	0,430 b
EX23	0,483 d	0,527 c	0,720 a	0,477 cd	0,257 a	0,457 bc	0,513 c	0,580 a	0,557 a
EX27	0,507 cd	0,473 d	0,380 cd	0,433 d	0,360 a	0,420 c	0,583 b	0,577 a	0,547 ab
Pr > F(Model)	<0,0001	<0,0001	<0,0001	<0,0001	0,093	<0,0001	<0,0001	0,049	0,009
Significate	Yes	Yes	Yes	Yes	Non	Yes	Yes	Yes	Yes
Pr > F(PURE DO3)	<0,0001	<0,0001	<0,0001	<0,0001	0,093	<0,0001	<0,0001	0,049	0,009
Significant	Yes	Yes	Yes	Yes	Non	Yes	Yes	Yes	Yes

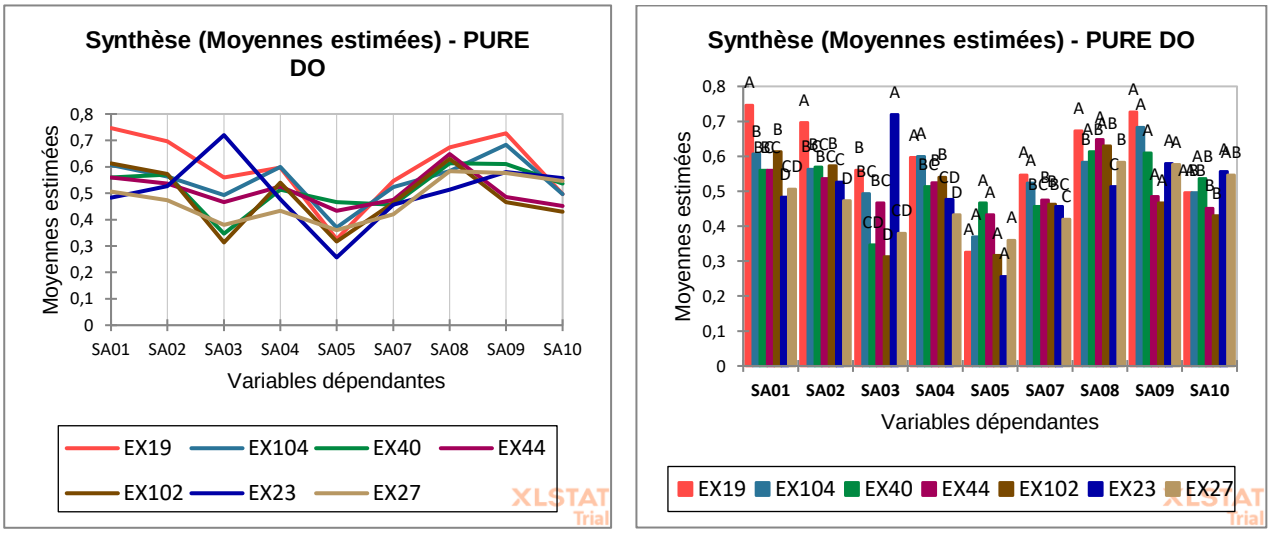


Figure 19: Effect of the crude extract against *S. aureus* biofilm formation

In the interaction between *S. aureus* and the crud extract, the result demonstrated that the top score anti-biofilm activity was of the EX104 (of D16) against *S. aureus* strain SA09 and 10 (from OD_{630nm} = 0.73 to OD_{630nm} = 0.30), while the other crude extract have a moderate closed activity against *S. aureus* strains.

Furthermore, the EX104 (of D16) play the major anti-biofilm against six *E. coli* strains (EC: 01, 04, 06, 07, 08 and 10), the best results is against *E. coli* 08 (from OD_{630nm} = 0.74 to OD_{630nm} = 0.52), while the highest anti-biofilm activity was Ex115 (of D47) against *E. coli* 02 (from OD_{630nm} = 0.53 to OD_{630nm} = 0.26) as showed in (**fig. 20, tab. 08**).

Table 8: Effect of the crude extracts on *E.coli* biofilm formation

Modality	Ec01	Ec02	Ec03	Ec04	Ec06	Ec07	Ec08	Ec09	Ec10
PURE DO3	0,500 ab	0,540 b	0,680 a	0,700 a	0,750 a	0,800 a	0,730 a	0,640 a	0,630 ab
PURE DO2	0,480 b	0,540 b	0,650 a	0,670 a	0,720 a	0,720 b	0,730 a	0,600 bcd	0,640 ab
PURE DO1	0,490 b	0,510 b	0,660 a	0,690 a	0,740 a	0,410 f	0,750 a	0,620 abc	0,670 a
EX23	0,567 a	0,417 c	0,610 b	0,550 c	0,580 c	0,567 cd	0,617 b	0,630 ab	0,627 ab
EX44	0,493 b	0,520 b	0,563 c	0,513 d	0,623 b	0,513 e	0,540 d	0,590 cd	0,620 ab
EX27	0,470 b	0,370 d	0,610 b	0,580 b	0,593 bc	0,550 d	0,613 b	0,590 cd	0,577 bc
EX115	0,393 c	0,613 a	0,257 d	0,543 c	0,583 bc	0,590 c	0,587 c	0,563 e	0,580 b
EX104	0,463 b	0,523 b	0,080 e	0,493 d	0,560 c	0,490 e	0,520 d	0,587 d	0,513 c
Pr > F(Model)	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Significat	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pr > F(DO/STRAINS)	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

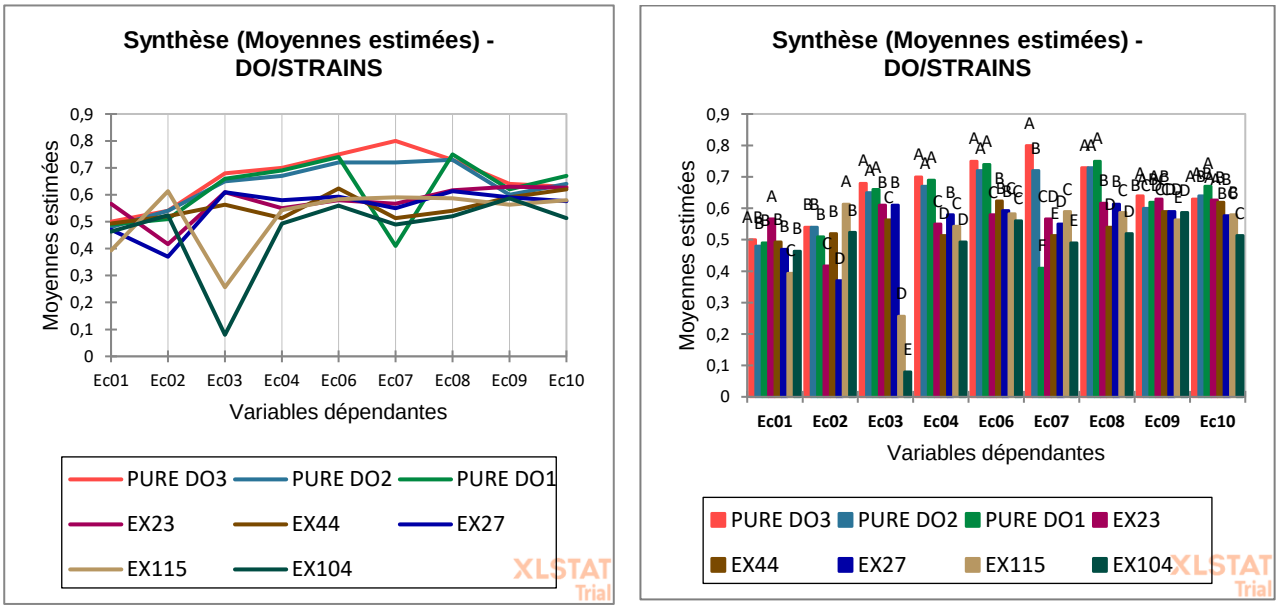


Figure 20: Effect of the crude extract against *E.coli* biofilm formation

The two *P. aeruginosa* strains 01 and 09 were their biofilm formation was affected by the crude extract EX27 (of D35) (from OD= 0.66 to OD= 0.38) and (from OD= 0.60 to OD= 0.41) respectively as described in (**fig.19, tab. 10**).

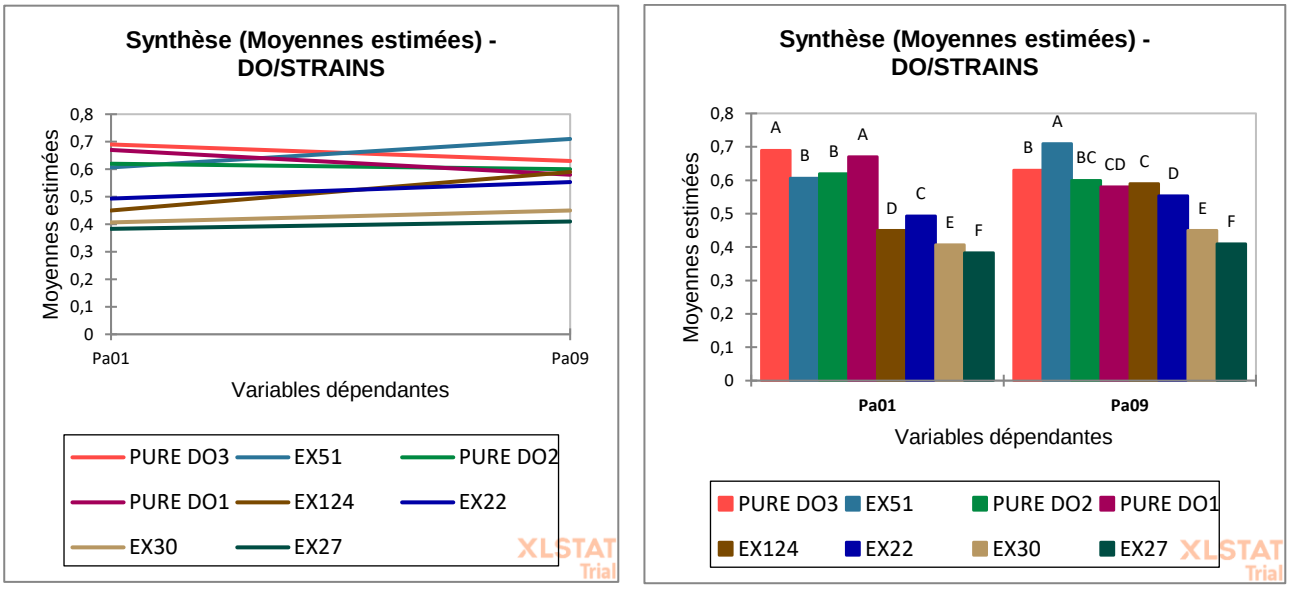


Figure 21: effect of the crude extract against *P. aeruginosa* biofilm formation

It should be noted that the crude extract EX44 and EX104 showed an anti-biofilm activity against both of S. *aureus* and *E.coli* strains, while EX27 has the largest ant-biofilm spectrum against the three clinical strains, all the crude extract with concrete results.

2.4. Taxonomic study of the selected Actinobacteria isolates:

According to the RNA 16 s molecular identification, the results of 24 strains of Actinobacteria were affiliated to four clusters, belonging to five different genera; including Streptomyces, Nocardiopsis,

Micromonospora, Actinomadura, Cellulomonas with the largest fraction of the isolates being assigned to the genera Streptomyces. The results are shown in **Table 9**

Table 9: Molecular identification of Actinobacteria isolates

Query of	Genus	Closest types species	Similarity%
D42	Genus I	*Streptomyces mutabilis*|NBRC 12800|AB184156^T	88.1
D36		*Streptomyces djakartensis*|NBRC 15409|AB184657^T	97.49
D27		*Streptomyces asenjonii*|KNN 35.1b|LT621750^T	91.67
D35		*Streptomyces gossypiisoli*|TRM 44567|MN548415^T	97.06
D47		*Streptomyces bellus*|ISP 5185|AJ399476^T	91.51
D42		*Streptomyces smyrnaeus*|SM3501|KF006349^T	94.5
D22		*Streptomyces specialis*|GW41-1564|LN929789^T	92.15
D16		*Streptomyces coerulescens*|ISP 5146|AY999720^T	98.48
D30		*Streptomyces canarius*|NBRC 13431|AB184396^T	86.74
D11		*Streptomyces diastaticus*|*NBRC* 3714|AB184785^T	90.25
D26		*Streptomyces malachitospinus*|NBRC 101004|AB249954^T	73.23
D32		*Streptomyces atrovirens*|NRRL B-16357|DQ026672^T	97.95
D11		*Streptomyces chilikensis*|RC 1830|JN050256^T	90.25
D15		*Streptomyces apricus*|SUN51|MN133488^T	95.11
D45		*Streptomyces viridochromogenes*|NBRC 3113|AB184728^T	97.33
D11		*Streptomyces fragilis*|NRRL 2424|AY999917^T	90.25
D48		*Streptomyces azureus*|ATCC 14921|DF968281^T	98.13
D21	Genus II	Nocardia cyriacigeorgica|DSM 44484|AF430027^T	96.81
D39	Genus III	*Micromonospora globbae*|WPS1-2|LC177396^T	88.59
D12		*Micromonospora tulbaghiae*|DSM 45142|jgi.1058868^T	97.67
D38	Genus IV	*Actinomadura fibrosa*|ATCC 49459|AF163114^T	94.24
D18	Genus V	*Cellulomonas telluris*|CPCC 204705|QXFN01000007^T	98.03

The evolutionary history was inferred using the Neighbour-Joining method (Saitou & Nei, 1987). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004), and are in the units of the number of base substitutions per site. This analysis involved 46 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 711 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

Evolutionary relationships of the isolated Actinobacteria strains can be observed in the 16S rRNA gene phylogenetic tree presented in **fig**. **22**. The treatment methods and culture media employed for the isolation of Actinobacteria seem to have selected various isolates of different species as many of them were found to group very closely or diversely, especially those affiliated with the genus.

- **Genus I:**

This cluster is the largest group; include 23 isolates of Actinobacteria, which belong all to the genus of Streptomyces. This result is consistent with previous studies, which reported that Streptomyces was the major genus of Actinobacteria in the soil (Rammali et al., 2024; Xu et al., 1996).

Ten isolates, D26, D30, D38, and D42 were assigned, with the high bootstrap values 100, to the type strains belong to the genus Streptomyces. While the percentage of similarity for the 17 isolates affiliated to the genus Streptomyces, range from 73.23% for the strain D26 with *Streptomyces malachitospinus*, to 98.48% for the isolate D11 with *Streptomyces coerulescens.*

All strains belong to this cluster form branched substrate mycelium, which is rarely fragmented. At maturity, the aerial mycelium forms chains of 3–20 spores (Bhowmick et al., 2024; Flärdh & Buttner, 2009). Members of the genus Streptomyces represent the primary source of secondary metabolites from the microbial origin (Krysenko, 2024; Krysenko & Wohlleben, 2024). Indeed, according to the database 'dictionary of natural products' (CRC press; Taylor and Francis group), 7953 molecules have been isolated from this genus (Messaoudi et al., 2020). For that reason, the probability of obtaining new compounds from the genus Streptomyces has become increasingly low, due to the mechanism of genetic exchange between the strains in the environment, consequently, the actual trend is oriented towards exploiting secondary metabolisms of rare Actinobacteria (Doroghazi & Buckley, 2010; Hopwood, 2019).

- **Genus II:**

This cluster is represented by one isolate, which belong to the genus of Nocardiopsis Indeed, the species belongs to the genus Nocardiopsis are known for their tolerances to high NaCl concentrations, and they are abundant in the saline areas (Bennur et al., 2015; Boudjelal et al., 2023). The isolates belongs to this cluster form a dense and branched, well developed substrate mycelium which fragments, at maturity, into rod-shaped and non-motile spores; however, the aerial mycelium breaks up into chains of straight, branched, or zigzag spores. This microscopic morphology typically characterizes the genus Nocardiopsis (Xu et al., 1996).

According to the molecular identification, the isolated strain, are close to the specie *Nocardia cyriacigeorgica*|DSM 44484|AF430027 with similarity values 96.81%. However the Actinobacteria strains belonging to the genius Nocardepsis isolated by Messaoudi, T14 and A58, has a similarity of 100% with the species *Nocardiopsis halotolerans and Nocardiopsis arvandica* respectively. The strain CG3 show low similarity (99.20%) with the species *Nocardiopsis rosea* (Messaoudi et al., 2020).Whereas, *Nocardiopsis alba* is the most isolated strain by Gohel using different growth conditions and methods, it should be notes that the use of conventional methods and molecular approaches can be led directly to the distinct species (Gohel & Singh, 2018).

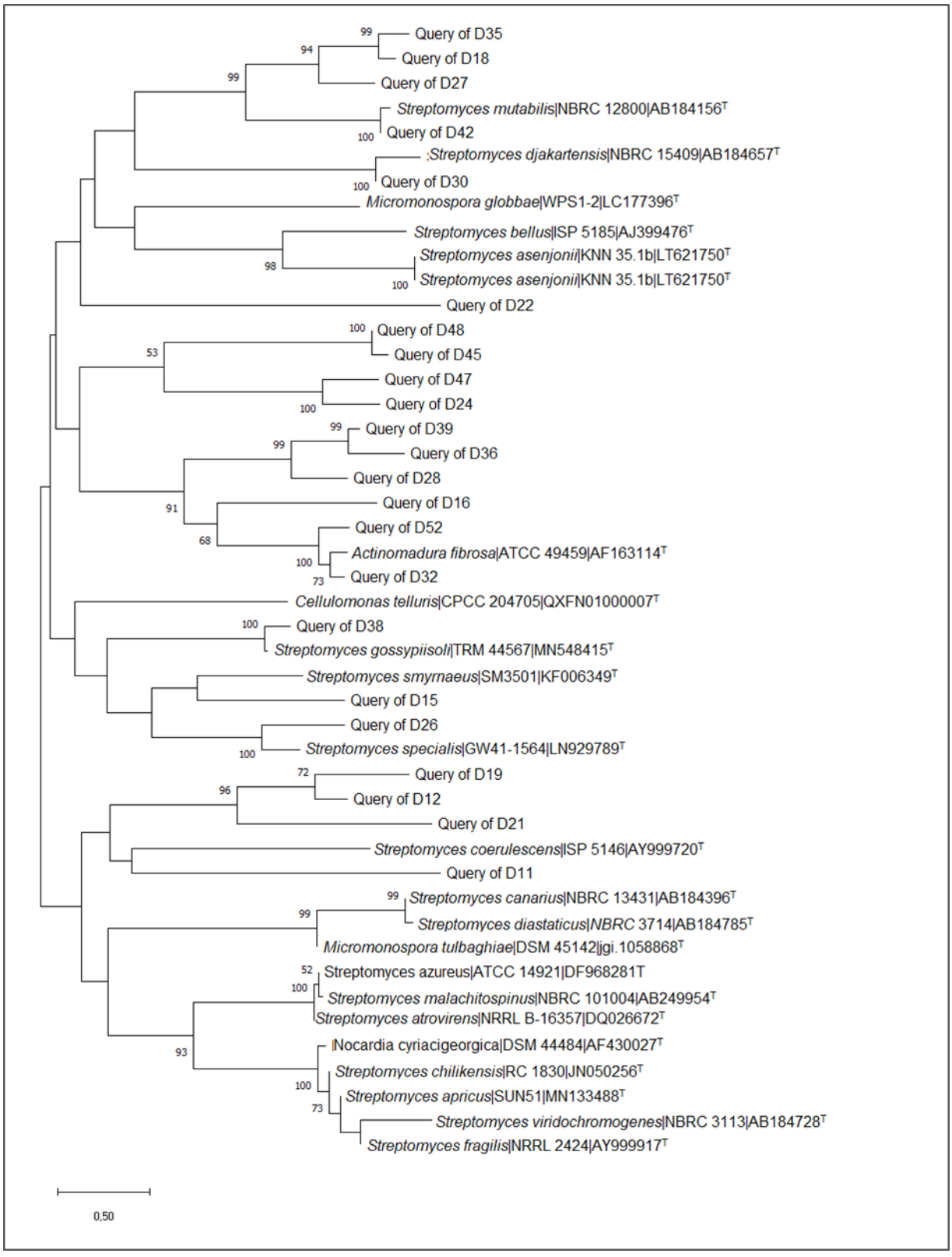


Figure 22: Phylogenetic tree of the Isolated Actinobacteria genera

Phylogenetic tree of the Actinobacteria strains isolated from *Acacia Senegal* rhizosphere and their GenBank nearest neighbours with similarity < 98.4. Maximum likelihood phylogenetic tree was performed with MEGA 11 using 24 sequences with 1090 bp. The phylogeny test used was the bootstrap method with 1000 replications. Bootstrap values shown at nodes support the branching order of the tree.

- **Genus III:**

This cluster is represented by two isolates, D12 and D39, which belong all to the family of Micromonosporaceae. These strains were isolated from the OUTOUL and ABALISSA Acacia rhizosphere soil respectively. Molecular identification indicates that the strain D12 was close to the specie *Micromonospora tulbaghiae|DSM 45142|jgi.1058868* with 97.67% similarity, while the isolate D39 was close to the species *Micromonospora globbae|WPS1-2|LC177396* with 88.59% similarity. Phylogenetic tree, Figure 1, indicate that the strain D 12 and D39, form a distinct branch within the cluster formed by the Streptomyces species.

- **Genus IV:**

This cluster is represented by one isolate D38. Sequencing of 16S rDNA indicates that the isolate D38 was close to *Actinomadura fibrosa |ATCC 49459|AF163114T* with 94.24%, the genera Actinomadura, is a member of the family Thermomonosporaceae. The strains was isolated from ABALISSA's Acacia trees. The strains D38 showed macroscopic and microscopic characteristics typical of the species belongs to the genus Actinomadura.

- **Genus V:**

This cluster including one strains Cellulomonas telluris|CPCC 204705|QXFN01000007T, this latter contains a heterogeneous collection of cellulose-decomposing bacteria principally isolated from soil materials, which produce various cellulose degrading enzymes under natural conditions. The genus Cellulomonas along with Jonesia, Oerskovia and Promicromonospora has been assigned to a new family Cellulomonadaceae. Phylogenetically the family belongs to the order Actinomycetales (Lv et al., 2022; Rajoka, 1999)

- **Genus VI:**

Tow strains were included in this cluster D 39 and D12 belonging to the genius Micromonospora were closed to the species *Micromonospora globbae*|WPS1-2|LC177396 and *Micromonospora tulbaghiae*|DSM 45142|jgi.1058868 with similarity of 88.59% and 97.67% respectively. Until the year 2018 *Micromonospora globbae* considered as sp. nov., an endophytic Actinomycete isolated by Kuncharoen from the roots of *Globba winitii C. H. Wright* tree (Kuncharoen et al., 2018).

The situation at the genus level is not dissimilar to that outlined above as 16S rRNA gene trees often also lack the resolution to distinguish between closely related genera, as recorded by (Nouioui et al., 2018).

On the other side, at the level of species six Actinobacteria strains D4, D14, D25, D31, D33 and D40 were achieved the similarity higher than 98.6% (W.-J. Li et al., 2024) to be close to five distinct species as described in **fig. 23**. The phylogenetic tree represent a bootstrap of 100 between the isolate D4 and the type strain *Streptomyces lomondensis*|NBRC 15426|AB184673. Furthermore, the strains D14, D40 and D45 are closed to the specie *Streptomyces tuirus*|NBRC 15617|AB184690, with bootstrap 85 and 98, while the phylogenetic analysis of the isolates assigned to the genius

Streptomyces showed that the Actinobacteria strain D31 and D33 formed a separate branch in the phylogenetic tree.

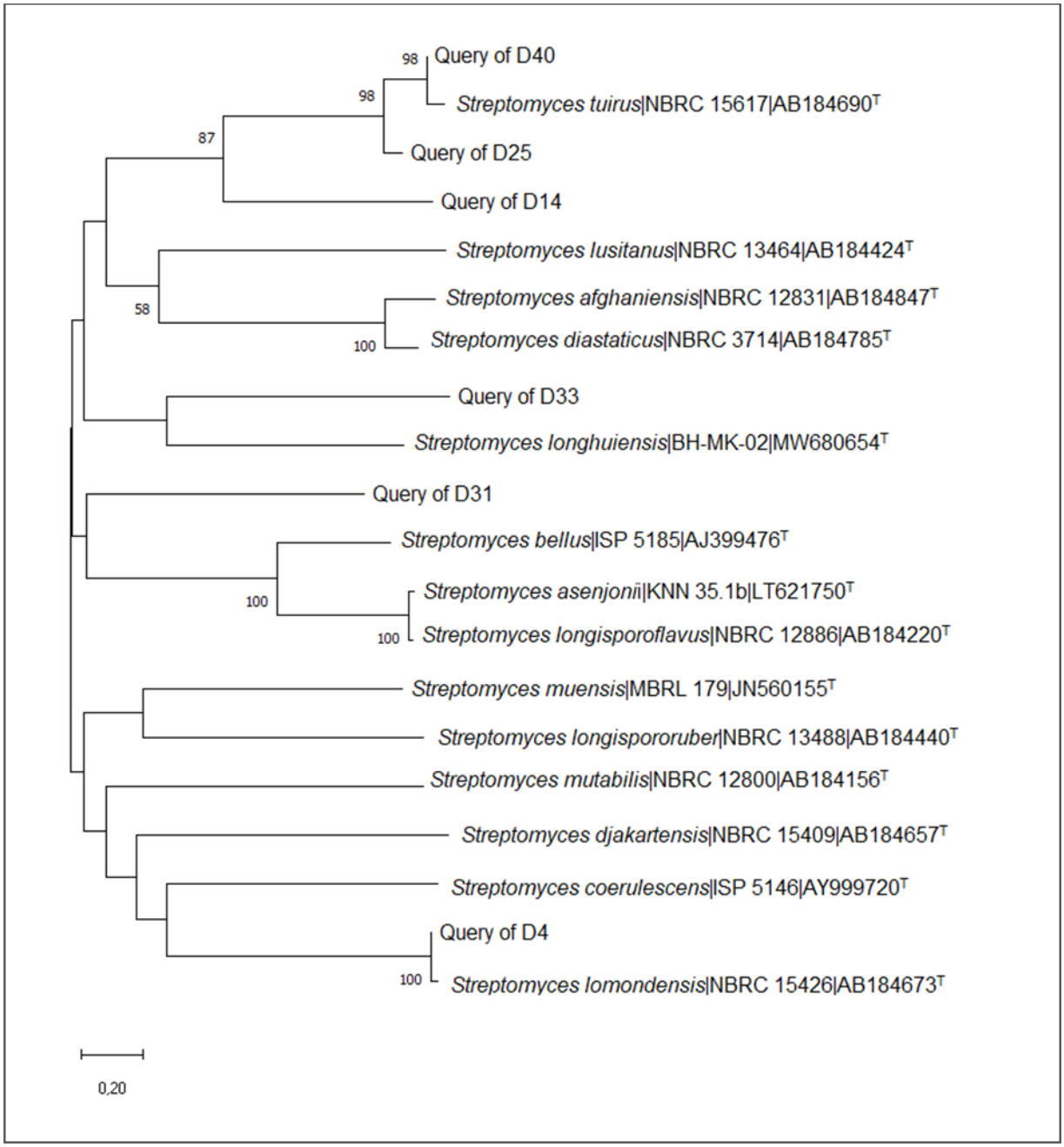


Figure 23: Phylogenetic tree of Isolated Actinobacteria species

Phylogenetic tree of the Actinobacteria strains isolated from Acacia Senegal rhizosphere and their GenBank nearest neighbours with similarity >98.4. Maximum likelihood phylogenetic tree was performed with MEGA 11 using 24 sequences with 1090 bp. The phylogeny test used was the bootstrap method with 1000 replications. Bootstrap values shown at nodes support the branching order of the tree.

CONCLUSION

Conclusion and Perspectives:

Thus, in view of the current emergence of new resistance gene to antibiotic associated with virulence genes in clinical bacterial strains that complicate the therapeutic treatment of the patients. The search for new ecosystems for the isolation of Actinobacteria is crucial for the discovery of new species and/or new natural bioactive substances non-toxic to the host and owed with antibacterial and anti-biofilm activity.

The initial motivation of this research work concerns the study of the qualitative and quantitative phenotypic of the formation of biofilms, as well as monitoring the kinetics of this formation by three clinical strains *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.,* previously isolated from different types of clinical samples. The results showing that the formation of biofilms is dependents on the conditions and the method of growth, where the CRA method is considered as the faster selective phenotypic method that give reflect about the ability to produce the slim, about 10% of the isolates express as strong producers, 30% are moderate and 50% are weakly producers. While qualitative evaluation of biofilm production by the TAM method, in tubes and after 24 hours of incubation, revealed that *E.coli* strains and *P. aeruginosa* strains are the most strains form a ring inside the test tube and considered as a higher productive of biofilm. The greater proportions of fixed cells of the strains *E.coli* 07, 08 and 10 were recorded in BHIB, while the two *P. aeruginosa* are highly adherent. However no adherents *S. aureus* strains by TAM method.

Furthermore, the standard CV staining method MTP, applied in a 96-well microplate, has made it possible to demonstrate the capacity of all isolated strains to form biofilms after 24 hours of incubation with constant intensities of CV staining 0.5%. Indeed, the strains *S. aureus* 01, 02 and *09, E coli* 06 and 08 were the highest productive with OD_{630nm} = 0.75, 0.74, 0.73 and 0.74, 0.74 respectively, the other strains are in average of OD_{630nm} = 0.49 to 0.69, while the *S. aureus* 05 strain was weakly biofilm-forming with OD_{630nm} =0.33.

Concerning on the natural source as biological control of biofilm, Actinobacteria was the subjected strains. Although, seven rhizospheric soil of Acacia tree from seven sites were explored on their productivity of Actinobacteria using dependent culture on Glycerol Yeast extract Agar. In fact, 124 strain were obtained, their macroscopic and microscopic characterization seems to Actinobacteria strains with diversity in their macroscopic consistency.

The molecular analysis and the identification of the RNA 16s, 24 Actinobacteria strains were belonging to five different genera; including Streptomyces, Nocardiopsis, Micromonospora, Actinomadura, Cellulomonas with the largest fraction of the isolates being assigned to the

genera Streptomyces. The phylogenic tree was affiliated the identified strains to four clusters in the level of genus which have a similarity low than 98.6%, while at the level of species only six Actinobacteria strains D4, D14, D25, D31, D33 and D40. Theses strains were achieved the similarity higher than 98.6% to be closed to five distinct species: *Streptomyces lomondensis*|NBRC 15426|AB184673, *Streptomyces tuirus*|NBRC 15617|AB184690, *Streptomyces bellus*|ISP 5185|AJ399476T and *Streptomyces longhuiensis*|BH-MK-02|MW680654^{T}.

The direct interaction between Actinobacteria and clinical strains realised by cross streak method revealed that the majority of the isolated Actinobacteria were extremely active against all *S. aureus* strains as Gram-positive bacteria, with inhibition zone ≤ 35 mm, the Actinobacteria strains D25, D32 and D48 showed a selective activity against all *S. aureus* strains with average of inhibition zone (mm) varied [4-20], [2-8] and ≤ 4 respectively. The antagonistic activity were decreased in the interaction in contact with *E. coli*, with inhibition zone ≤ 25 mm, where the Actinobacteria strains D24 and D42, have a selective activity only against *E. coli* strains (all tested strains) with inhibition zone [6-20], [5-11] respectively. Whilst, *P .aeruginosa* strains were highly resisted in the antagonistic interaction with Actinobacteria strains.

In contrast, The Actinobacteria strains D31, D33, D35, D36 and D47 have been registered the largest antagonistic activity [3-6], [2-5], [20-35], [20-34] and [7-15] respectively against all *S. aureus* strains, as well as against all *E.coli* strains [5-11], [2-7], [18-25], [4-6] and[4-20] respectively. It is necessary to indicate that the strain D35 are the best isolate on their antagonistic activity. Nevertheless, no antagonistic activity have recorded by the strains D1, D11, D12, D14, D21, D22, D26, D27, D28, D30, D32, D38, D39, D40 and D45.

On the other side, the biofilm biocontrol assays between the Actinobacteria crude extract and the clinical strains have revealed that the majority of the crud extract have a significant effect on the production of biofilms. Whereas, the top score anti-biofilm activity was of the EX104 against *S. aureus* 09 and 10 (from OD_{630nm} = 0.73 to OD_{630nm} = 0.30), followed by Ex115 against *E.coli* 02 (from OD_{630nm} = 0.53 to OD_{630nm} = 0.26). Meanwhile, the EX 104 play the major anti-biofilm against six *E.coli* strains: 01, 04, 06, 07, 08 and 10, the best results is against *E.coli* 08 (from OD_{630nm} = 0.74 to OD_{630nm} = 0.52). Whilst, *P. aeruginosa* strains 01 biofilm formation was affected by the crude extract EX27 (from OD_{630nm} = 0.66 to OD_{630nm} = 0.38).

This scientific issue is complex and multifaceted, with various perspectives to consider for further future studies, and while it is tempting to conclude that the studied clinical strains are

single biofilm species producers, a closer examination reveals that the clinical strains have the ability to form a mixed biofilms inter them. In contrast, The bioactive compound and their encoded genes of Actinobacteria strains D31, D33, D35, D36 and D47 should be characterise and identified, and tested against the mixed biofilm of the three strains *Staphylococcus aureus., Pseudomonas aeruginosa* and *Escherichia coli.*

References:

Abdullahi, U. F., Igwenagu, E., Mu'azu, A., Aliyu, S., & Umar, M. I. (2016). Intrigues of biofilm: a perspective in veterinary medicine. *Veterinary World*, *9*(1), 12.

Abussaud, M. J., Alanagreh, L., & Abu-Elteen, K. (2013). Isolation, characterization and antimicrobial activity of Streptomyces strains from hot spring areas in the northern part of Jordan. *African Journal of Biotechnology*, *12*(51), 7124–7132.

Aggarwal, N., Kitano, S., Puah, G. R. Y., Kittelmann, S., Hwang, I. Y., & Chang, M. W. (2022). Microbiome and human health: Current understanding, engineering, and enabling technologies. *Chemical Reviews*, *123*(1), 31–72.

Al-Ansari, M., Kalaiyarasi, M., Almalki, M. A., & Vijayaraghavan, P. (2020). Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from Streptomyces sp. AS11 isolated from the marine environment. *Journal of King Saud University-Science*, *32*(3), 1993–1998.

Amran, S. S. D., Syaida, A. A. R., Jalil, M. T. M., Nor, N. H. M., & Yahya, M. F. Z. R. (2024). Preparation of Biofilm Assay Using 96-Well and 6-Well Microplates for Quantitative Measurement and Structural Characterization: A Review. *Science Letters*, *18*(2), 121–134.

Antunes, N. T., Lamoureaux, T. L., Toth, M., Stewart, N. K., Frase, H., & Vakulenko, S. B. (2014). Class D β-lactamases: are they all carbapenemases? *Antimicrobial Agents and Chemotherapy*, *58*(4), 2119–2125.

Archer, N. K., Mazaitis, M. J., Costerton, J. W., Leid, J. G., Powers, M. E., & Shirtliff, M. E. (2011a). *Properties , regulation and roles in human disease Staphylococcus aureus biofilms*. *October*, 445–459.

Archer, N. K., Mazaitis, M. J., Costerton, J. W., Leid, J. G., Powers, M. E., & Shirtliff, M. E. (2011b). Staphylococcus aureus biofilms: properties, regulation, and roles in human disease. *Virulence*, *2*(5), 445–459.

Arunachalam, K., & Davoodbasha, M. (2021). Imaging bacteria and biofilm by field emission scanning electron microscopy. *Analytical Methodologies for Biofilm Research*, 205–222.

Arunachalam, K., Pandurangan, P., Shi, C., & Lagoa, R. (2023). Regulation of

Staphylococcus aureus virulence and application of nanotherapeutics to eradicate s. aureus infection. *Pharmaceutics*, *15*(2), 310.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., & Qamar, M. U. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*, *11*, 1645.

Asnani, A., Purwanti, A., Bakrudin, W. A., & Anjarwati, D. U. (2022). The Production of Streptomyces W-5B Extract for Antibiofilm against Methicillin-resistant Staphylococcus aureus. J Pure Appl Microbiol. 2022; 16 (1): 337-346. doi: 10.22207/JPAM. 16.1. 23 The Author (s) 2022. *Open Access. This Article Is Distributed under the Terms of the Creative Commons Attribution*, *4*.

Assefa, M., & Amare, A. (2022). Biofilm-Associated multi-drug resistance in hospital-acquired infections: a review. *Infection and Drug Resistance*, 5061–5068.

Augustine, N., Peter A, W., Kerkar, S., & Thomas, S. (2012). Arctic actinomycetes as potential inhibitors of Vibrio cholerae biofilm. *Current Microbiology*, *64*, 338–342.

Avila-Novoa, M.-G., Iñíguez-Moreno, M., Solís-Velázquez, O.-A., González-Gomez, J.-P., Guerrero-Medina, P.-J., & Gutiérrez-Lomelí, M. (2018). Biofilm formation by Staphylococcus aureus isolated from food contact surfaces in the dairy industry of Jalisco, Mexico. *Journal of Food Quality*, *2018*.

Azman, A.-S., Mawang, C.-I., Khairat, J.-E., & AbuBakar, S. (2019). Actinobacteria—a promising natural source of anti-biofilm agents. *International Microbiology*, *22*(4), 403–409.

Bakkiyaraj, D., & Karutha Pandian, S. T. (2010). In vitro and in vivo antibiofilm activity of a coral associated actinomycete against drug resistant Staphylococcus aureus biofilms. *Biofouling*, *26*(6), 711–717.

Balasubramanian, S., Othman, E. M., Kampik, D., Stopper, H., Hentschel, U., Ziebuhr, W., Oelschlaeger, T. A., & Abdelmohsen, U. R. (2017). Marine sponge-derived Streptomyces sp. SBT343 extract inhibits staphylococcal biofilm formation. *Frontiers in Microbiology*, *8*, 236.

Ballén, V., Cepas, V., Ratia, C., Gabasa, Y., & Soto, S. M. (2022). Clinical Escherichia coli: from biofilm formation to new antibiofilm strategies. *Microorganisms*, *10*(6), 1103.

REFERENCES

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79.

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., & van Wezel, G. P. (2016a). Taxonomy, physiology, and natural products of Actinobacteria. *Microbiology and Molecular Biology Reviews*, *80*(1), 1–43.

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., & van Wezel, G. P. (2016b). Taxonomy, Physiology, and Natural Products of Actinobacteria *Microbiology and Molecular Biology Reviews*, *80*(1), 1 LP – 43. https://doi.org/10.1128/MMBR.00019-15

Belgacem, H., Benreguieg, M., Benabbou, T. A., & Khoula, R. (2023). SCREENING OF NOVEL STREPTOMYCES SP. TR10 FROM THE RHIZOSPHERE OF ACACIA IN THE ALGERIAN DESERT AND EVALUATION OF THEIR ANTAGONISTIC POTENTIAL. *Growth*, *3*, 4.

Bennur, T., Kumar, A. R., Zinjarde, S., & Javdekar, V. (2015). Nocardiopsis species: Incidence, ecological roles and adaptations. *Microbiological Research*, *174*, 33–47.

Benyamini, P. (2024). Beyond Antibiotics: What the Future Holds. *Antibiotics*, *13*(10), 919.

Berger, D., Rakhamimova, A., Pollack, A., & Loewy, Z. (2018). Oral biofilms: development, control, and analysis. *High-Throughput*, *7*(3), 24.

Bergey, D. H. (1994). *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins.

Bhowmick, S., Viveros, R. P., Latoscha, A., Commichau, F. M., Wrede, C., Al-Bassam, M. M., & Tschowri, N. (2024). Cell shape and division septa positioning in filamentous Streptomyces require a functional cell wall glycopolymer ligase CglA. *Mbio*, e01492-24.

Bhowmik, A., Malhotra, A., Jana, S., & Chauhan, A. (2021). Biofilm Formation and Pathogenesis. *Analytical Methodologies for Biofilm Research*, 3–37.

Boles, B. R., Thoendel, M., Roth, A. J., & Horswill, A. R. (2010). Identification of genes

involved in polysaccharide-independent Staphylococcus aureus biofilm formation. *PloS One*, *5*(4), e10146.

Boudjelal, F., Zitouni, A., Bouras, N., Spröer, C., Klenk, H.-P., Smaoui, S., & Mathieu, F. (2023). Rare Halophilic Nocardiopsis from Algerian Saharan Soils as Tools for Biotechnological Processes in Pharmaceutical Industry. *BioMed Research International*, *2023*(1), 1061176.

Boughachiche, F., Reghioua, S., Zerizer, H., & Boulahrouf, A. (2012). Activité antibactérienne d'espèces rares de Streptomyces contre des isolats cliniques multirésistants. *Annales de Biologie Clinique*, *70*(2), 169–174.

Bouras, N., Yekkour, A., Smaoui, S., Mellouli, L., & Barakate, M. (2021). Actinobacterial Abundance and Interrelationships in Ecosystems of Northwest Africa. In *Microbes in Microbial Communities* (pp. 337–370). Springer.

Bueno, J. (2014). Anti-biofilm drug susceptibility testing methods: looking for new strategies against resistance mechanism. *Journal of Microbial & Biochemical Technology S*, *3*(2).

Bueno, Juan, & Bueno, J. (2020). Antimicrobial Nanotechnology in Preventing the Transmission of Infectious Disease. *Preclinical Evaluation of Antimicrobial Nanodrugs*, 75–88.

Bush, K., & Bradford, P. A. (2020). Epidemiology of β-lactamase-producing pathogens. *Clinical Microbiology Reviews*, *33*(2), e00047-19.

Butrico, C. E., & Cassat, J. E. (2020). *Quorum Sensing and Toxin Production in Staphylococcus aureus Osteomyelitis: Pathogenesis and Paradox. Toxins 12, 516*.

Bystrianský, L., Hujslová, M., Hršelová, H., Řezáčová, V., Němcová, L., Šimsová, J., Gryndlerová, H., Kofroňová, O., Benada, O., & Gryndler, M. (2019). Observations on two microbial life strategies in soil: Planktonic and biofilm-forming microorganisms are separable. *Soil Biology and Biochemistry*, *136*, 107535.

Calderaro, A., & Chezzi, C. (2024). MALDI-TOF MS: A Reliable Tool in the Real Life of the Clinical Microbiology Laboratory. *Microorganisms*, *12*(2), 322.

Carroll, K. C., Borek, A. P., Burger, C., Glanz, B., Bhally, H., Henciak, S., & Flayhart, D. C. (2006). Evaluation of the BD Phoenix automated microbiology system for

identification and antimicrobial susceptibility testing of staphylococci and enterococci. *Journal of Clinical Microbiology*, *44*(6), 2072–2077.

Chandki, R., Banthia, P., & Banthia, R. (2011). Biofilms: A microbial home. *Journal of Indian Society of Periodontology*, *15*(2), 111–114.

Chang, H.-H., Cohen, T., Grad, Y. H., Hanage, W. P., O'Brien, T. F., & Lipsitch, M. (2015). Origin and proliferation of multiple-drug resistance in bacterial pathogens. *Microbiology and Molecular Biology Reviews*, *79*(1), 101–116.

Characklis, W. G. (1973). Attached microbial growths-II. Frictional resistance due to microbial slimes. *Water Research*, *7*(9), 1249–1258.

Chavant, P., Gaillard-Martinie, B., Talon, R., Hébraud, M., & Bernardi, T. (2007). A new device for rapid evaluation of biofilm formation potential by bacteria. *Journal of Microbiological Methods*, *68*(3), 605–612.

Cheesebrough, M. (1998). District laboratory practice in tropical countries, part II (Microbiology). *Cambridgeshire Tropical Health Technology, Cambridge, UK*, *231*.

Christensen, G. D., Simpson, W. A., Bisno, A. L., & Beachey, E. H. (1982). Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. *Infection and Immunity*, *37*(1), 318–326.

Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M., & Beachey, E. H. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *Journal of Clinical Microbiology*, *22*(6), 996–1006.

Ciandrini, E., Morroni, G., Cirioni, O., Kamysz, W., Kamysz, E., Brescini, L., Baffone, W., & Campana, R. (2020). Synergistic combinations of antimicrobial peptides against biofilms of methicillin-resistant Staphylococcus aureus (MRSA) on polystyrene and medical devices. *Journal of Global Antimicrobial Resistance*, *21*, 203–210.

Clark, D. P., Pazdernik, N. J., & McGehee, M. R. (2019). *Chapter 23 - Plasmids* (D. P. Clark, N. J. Pazdernik, & M. R. B. T.-M. B. (Third E. McGehee (eds.); pp. 712–748). Academic Cell. https://doi.org/https://doi.org/10.1016/B978-0-12-813288-3.00023-9

Coenye, T., Bové, M., & Bjarnsholt, T. (2022). Biofilm antimicrobial susceptibility through an experimental evolutionary lens. *Npj Biofilms and Microbiomes*, *8*(1), 82.

REFERENCES

Coffey, B. M., & Anderson, G. G. (2014). Biofilm formation in the 96-well microtiter plate. *Pseudomonas Methods and Protocols*, 631–641.

Colvin, K. M., Irie, Y., Tart, C. S., Urbano, R., Whitney, J. C., Ryder, C., Howell, P. L., Wozniak, D. J., & Parsek, M. R. (2012). The Pel and Psl polysaccharides provide Pseudomonas aeruginosa structural redundancy within the biofilm matrix. *Environmental Microbiology*, *14*(8), 1913–1928.

Cordovez, V., Carrion, V. J., Etalo, D. W., Mumm, R., Zhu, H., Van Wezel, G. P., & Raaijmakers, J. M. (2015). Diversity and functions of volatile organic compounds produced by Streptomyces from a disease-suppressive soil. *Frontiers in Microbiology*, *6*, 1081.

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., & Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science*, *326*(5960), 1694–1697.

Costerton, J. W., Geesey, G. G., & Cheng, K.-J. (1978). How bacteria stick. *Scientific American*, *238*(1), 86–95.

Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science*, *284*(5418), 1318–1322.

Cue, D., Lei, M. G., Luong, T. T., Kuechenmeister, L., Dunman, P. M., O'Donnell, S., Rowe, S., O'Gara, J. P., & Lee, C. Y. (2009). Rbf promotes biofilm formation by Staphylococcus aureus via repression of icaR, a negative regulator of icaADBC. *Journal of Bacteriology*, *191*(20), 6363–6373.

Dai, L., Liu, C., Peng, L., Song, C., Li, X., Tao, L., & Li, G. (2021). Different distribution patterns of microorganisms between aquaculture pond sediment and water. *Journal of Microbiology*, *59*, 376–388.

Dang, H., & Lovell, C. R. (2016). Microbial Surface Colonization and Biofilm Development in Marine Environments. *Microbiology and Molecular Biology Reviews*, *80*(1), 91–138. https://doi.org/10.1128/mmbr.00037-15

Dekaboruah, E., Suryavanshi, M. V., Chettri, D., & Verma, A. K. (2020). Human microbiome: an academic update on human body site specific surveillance and its possible role. *Archives of Microbiology*, *202*, 2147–2167.

REFERENCES

Delbari, Y., Mohassel, Y., Kakaei, E., & Bahrami, Y. (2023). Identification and anti-bacterial property of endophytic actinobacteria from Thymes kotschyanus, Allium hooshidaryae, and Cerasus microcarpa. *Scientific Reports*, *13*(1), 13145.

Dewachter, L., Fauvart, M., & Michiels, J. (2019). Bacterial heterogeneity and antibiotic survival: understanding and combatting persistence and heteroresistance. *Molecular Cell*, *76*(2), 255–267.

Dilip, C. V, Mulaje, S. S., & Mohalkar, R. Y. (2013). A review on actinomycetes and their biotechnological application. *International Journal of Pharmaceutical Sciences and Research*, *4*(5), 1730.

Dinda, A. P., Asnani, A., & Anjarwati, D. U. (2021). The Activities of Streptomyces W-5A as antibacterial and antibiofilm towards methicillin-resistant Staphylococcus aureus 2983. *Proceedings of the 1st Jenderal Soedirman International Medical Conference in Conjunction with the 5th Annual Scientific Meeting (Temilnas) Consortium of Biomedical Science Indonesia*, 109–115.

Djinni, I., Defant, A., Kecha, M., & Mancini, I. (2019). Actinobacteria derived from Algerian ecosystems as a prominent source of antimicrobial molecules. *Antibiotics*, *8*(4), 172.

Dong, C.-L., Che, R.-X., Wu, T., Qu, Q.-W., Chen, M., Zheng, S.-D., Cai, X.-H., Wang, G., & Li, Y.-H. (2023). New Characterization of Multi-Drug Resistance of Streptococcus suis and Biofilm Formation from Swine in Heilongjiang Province of China. *Antibiotics*, *12*(1), 132.

Doroghazi, J. R., & Buckley, D. H. (2010). Widespread homologous recombination within and between Streptomyces species. *The ISME Journal*, *4*(9), 1136–1143.

Dutta, B., Nag, M., Lahiri, D., & Ray, R. R. (2021). Analysis of biofilm matrix by multiplex fluorescence in situ hybridization (M-FISH) and confocal laser scanning microscopy (CLSM) during nosocomial infections. *Analytical Methodologies for Biofilm Research*, 183–203.

Ebrahimi, M. T., Hedayati, M. A., Pirlar, R. F., Mortazavi, N., Nazari, M., Ahmadi, A., Hemmati, J., & Erfani, Y. (2023). Investigation of the biofilm formation in extra-intestinal pathogenic Escherichia coli ST131 strains and its correlation with the presence of fimH, afa, and kpsMSTII genes. *Journal of Applied Genetics*, 367–373. https://doi.org/10.1007/s13353-023-00757-9

REFERENCES

Edwards, U., Rogall, T., Blöcker, H., Emde, M., & Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research*, *17*(19), 7843–7853.

Elbendary, A. A., Hessain, A. M., El-Hariri, M. D., Seida, A. A., Moussa, I. M., Mubarak, A. S., Kabli, S. A., Hemeg, H. A., & El Jakee, J. K. (2018). Isolation of antimicrobial producing actinobacteria from soil samples. *Saudi Journal of Biological Sciences*, *25*(1), 44–46.

Elias, F., Muddada, S., Muleta, D., & Tefera, B. (2022). Antimicrobial potential of Streptomyces spp. isolated from the rift valley regions of Ethiopia. *Advances in Pharmacological and Pharmaceutical Sciences*, *2022*.

Elias, S., & Banin, E. (2012). Multi-species biofilms: living with friendly neighbors. *FEMS Microbiology Reviews*, *36*(5), 990–1004.

Escobar-Muciño, E., Arenas-Hernández, M. M. P., & Luna-Guevara, M. L. (2022). Mechanisms of Inhibition of Quorum Sensing as an Alternative for the Control of E. coli and Salmonella. *Microorganisms*, *10*(5), 884.

Fahr, A.-M., Eigner, U., Armbrust, M., Caganic, A., Dettori, G., Chezzi, C., Bertoncini, L., Benecchi, M., & Menozzi, M. G. (2003). Two-center collaborative evaluation of the performance of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of Enterococcus spp. and Staphylococcus spp. *Journal of Clinical Microbiology*, *41*(3), 1135–1142.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, *39*(4), 783–791.

Flärdh, K., & Buttner, M. J. (2009). Streptomyces morphogenetics: dissecting differentiation in a filamentous bacterium. *Nature Reviews Microbiology*, *7*(1), 36–49.

François, P., Schrenzel, J., & Götz, F. (2023). Biology and Regulation of Staphylococcal Biofilm. *International Journal of Molecular Sciences*, *24*(6), 5218.

Fu, J., Qin, R., Zong, G., Liu, C., Kang, N., Zhong, C., & Cao, G. (2019). The CagRS Two-Component System Regulates Clavulanic Acid Metabolism via Multiple Pathways in Streptomyces clavuligerus F613-1. *Frontiers in Microbiology*, *10*, 244. https://doi.org/10.3389/fmicb.2019.00244

REFERENCES

Gajic, I., Kabic, J., Kekic, D., Jovicevic, M., Milenkovic, M., Mitic Culafic, D., Trudic, A., Ranin, L., & Opavski, N. (2022). Antimicrobial susceptibility testing: a comprehensive review of currently used methods. *Antibiotics*, *11*(4), 427.

Gao, M., Manos, J., Whiteley, G., & Zablotska-Manos, I. (2024). Antibiofilm Agents for the Treatment and Prevention of Bacterial Vaginosis: A Systematic Narrative Review. *The Journal of Infectious Diseases*, jiae134.

Ghazalibina, M., Morshedi, K., Farahani, R. K., Babadi, M., & Khaledi, A. (2019). Study of virulence genes and related with biofilm formation in Pseudomonas aeruginosa isolated from clinical samples of Iranian patients; A systematic review. *Gene Reports*, *17*, 100471.

Ghellai, L., Hassaine, H., Klouche, N., Khadir, A., Aissaoui, N., Nas, F., & Zingg, W. (2014). Detection of biofilm formation of a collection of fifty strains of Staphylococcus aureus isolated in Algeria at the University Hospital of Tlemcen. *Journal of Bacteriology Research*, *6*(1), 1–6.

Gloag, E. S., Fabbri, S., Wozniak, D. J., & Stoodley, P. (2020). Biofilm mechanics: Implications in infection and survival. *Biofilm*, *2*, 100017.

Gohel, S. D., & Singh, S. P. (2018). Molecular phylogeny and diversity of the salt-tolerant alkaliphilic actinobacteria inhabiting coastal Gujarat, India. *Geomicrobiology Journal*, *35*(9), 775–789.

Gondil, V. S., & Subhadra, B. (2023). Biofilms and their role on diseases. *BMC Microbiology*, *23*(1), 203.

Goodfellow, M., & Fiedler, H.-P. (2010). A guide to successful bioprospecting: informed by actinobacterial systematics. *Antonie van Leeuwenhoek*, *98*(2), 119–142. https://doi.org/10.1007/s10482-010-9460-2

Gowrishankar, S., Duncun Mosioma, N., & Karutha Pandian, S. (2012). Coral-associated bacteria as a promising antibiofilm agent against methicillin-resistant and-susceptible Staphylococcus aureus biofilms. *Evidence-Based Complementary and Alternative Medicine, 2012*.

Grossich, R., Lemos Vilches, M., Costa, C. S., & Pezzoni, M. (2023). Role of Pel and Psl polysaccharides in the response of Pseudomonas aeruginosa to environmental

challenges: oxidative stress agents (UVA, H2O2, sodium hypochlorite) and its competitor Staphylococcus aureus. *Microbiology*, *169*(2), 1301.

Gunjal, A., & Bhagat, D. S. (2022). Diversity of actinomycetes in Western Ghats. In *Microbial Diversity in Hotspots* (pp. 117–133). Elsevier.

Haque, M. M., Mosharaf, M. K., Haque, M. A., Tanvir, M. Z. H., & Alam, M. K. (2021). Biofilm formation, production of matrix compounds and biosorption of copper, nickel and lead by different bacterial strains. *Frontiers in Microbiology*, *12*, 615113.

Harika, K., Shenoy, V. P., Narasimhaswamy, N., & Chawla, K. (2020). Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. *Journal of Global Infectious Diseases*, *12*(3), 129.

He, J., Wang, A., Liu, G., Gao, J., Ali, T., & Han, B. (2014). Biofilm Formation and Biofilm-Associated Genes Assay of Staphylococcus aureus Isolated from Bovine Subclinical Mastitis in China. *Pakistan Veterinary Journal*, *34*(4).

Hennig, S., Wai, S. N., & Ziebuhr, W. (2007). Spontaneous switch to PIA-independent biofilm formation in an ica-positive Staphylococcus epidermidis isolate. *International Journal of Medical Microbiology*, *297*(2), 117–122.

Herrling, M. P., Lackner, S., Nirschl, H., Horn, H., & Guthausen, G. (2019). Recent NMR/MRI studies of biofilm structures and dynamics. *Annual Reports on NMR Spectroscopy*, *97*, 163–213.

Heukelekian, H., & Heller, A. (1940). Relation between food concentration and surface for bacterial growth. *Journal of Bacteriology*, *40*(4), 547–558.

Holden, E. R., Yasir, M., Turner, A. K., Charles, I. G., & Webber, M. A. (2022). Comparison of the genetic basis of biofilm formation between Salmonella Typhimurium and Escherichia coli. *Microbial Genomics*, *8*(11), 885.

Hopwood, D. A. (2019). Highlights of Streptomyces genetics. *Heredity*, *123*(1), 23–32.

Huan, Y., Kong, Q., Mou, H., & Yi, H. (2020). Antimicrobial peptides: classification, design, application and research progress in multiple fields. *Frontiers in Microbiology*, *11*, 2559.

Huemer, M., Mairpady Shambat, S., Brugger, S. D., & Zinkernagel, A. S. (2020). Antibiotic

resistance and persistence—Implications for human health and treatment perspectives. *EMBO Reports*, *21*(12), e51034.

Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: past, present and future. *Current Opinion in Microbiology*, *51*, 72–80.

Idrees, M., Sawant, S., Karodia, N., & Rahman, A. (2021). Staphylococcus aureus biofilm: Morphology, genetics, pathogenesis and treatment strategies. *International Journal of Environmental Research and Public Health*, *18*(14), 7602.

Irie, Y., Starkey, M., Edwards, A. N., Wozniak, D. J., Romeo, T., & Parsek, M. R. (2010). Pseudomonas aeruginosa biofilm matrix polysaccharide Psl is regulated transcriptionally by RpoS and post-transcriptionally by RsmA. *Molecular Microbiology*, *78*(1), 158–172.

Jansson, J. K., & Hofmockel, K. S. (2018). The soil microbiome—from metagenomics to metaphenomics. In *Current Opinion in Microbiology* (Vol. 43, pp. 162–168). https://doi.org/https://doi.org/10.1016/j.mib.2018.01.013

Jebril, N. M. T. (2020). Evaluation of two fixation techniques for direct observation of biofilm formation of Bacillus subtilis in situ, on Congo red agar, using scanning electron microscopy. *Veterinary World*, *13*(6), 1133.

Jefferson, K. K., Pier, D. B., Goldmann, D. A., & Pier, G. B. (2004). The teicoplanin-associated locus regulator (TcaR) and the intercellular adhesin locus regulator (IcaR) are transcriptional inhibitors of the ica locus in Staphylococcus aureus. *Journal of Bacteriology*, *186*(8), 2449–2456.

Jennings, L. K., Storek, K. M., Ledvina, H. E., Coulon, C., Marmont, L. S., Sadovskaya, I., Secor, P. R., Tseng, B. S., Scian, M., & Filloux, A. (2015). Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. *Proceedings of the National Academy of Sciences*, *112*(36), 11353–11358.

Jo, J., Price-Whelan, A., & Dietrich, L. E. P. (2022). Gradients and consequences of heterogeneity in biofilms. *Nature Reviews Microbiology*, *20*(10), 593–607.

Jones, C. J., & Wozniak, D. J. (2017). Congo red stain identifies matrix overproduction and is an indirect measurement for c-di-GMP in many species of bacteria. In *c-di-GMP*

Signaling (pp. 147–156). Springer.

Jones, H. C., Roth, I. L., & Sanders III, W. M. (1969). Electron microscopic study of a slime layer. *Journal of Bacteriology*, *99*(1), 316–325.

Jose, P. A., Maharshi, A., & Jha, B. (2021). Actinobacteria in natural products research: Progress and prospects. *Microbiological Research*, *246*, 126708.

Juhas, M. (2023). The World of Microorganisms. In *Brief Lessons in Microbiology: From the Origin of Life to Artificial Intelligence* (pp. 1–16). Springer.

Kabiri, F., Aghaei, S. S., Pourbabaee, A. A., Soleimani, M., & Komeili Movahhed, T. (2023). Antibiofilm and cytotoxic potential of extracellular biosynthesized gold nanoparticles using actinobacteria Amycolatopsis sp. KMN. *Preparative Biochemistry & Biotechnology*, *53*(3), 265–278.

Kaiser, T. D. L., Pereira, E. M., Dos Santos, K. R. N., Maciel, E. L. N., Schuenck, R. P., & Nunes, A. P. F. (2013). Modification of the Congo red agar method to detect biofilm production by Staphylococcus epidermidis. *Diagnostic Microbiology and Infectious Disease*, *75*(3), 235–239.

Kamarudheen, N., & Rao, K. V. B. (2019). Fatty acyl compounds from marine Streptomyces griseoincarnatus strain HK12 against two major bio-film forming nosocomial pathogens; an in vitro and in silico approach. *Microbial Pathogenesis*, *127*, 121–130.

Kamath, A., Shukla, A., & Patel, D. (2023). Quorum Sensing and Quorum Quenching: Two sides of the same coin. *Physiological and Molecular Plant Pathology*, *123*, 101927.

Kamil, F. H., Saeed, E. E., El-Tarabily, K. A., & AbuQamar, S. F. (2018). Biological control of mango dieback disease caused by Lasiodiplodia theobromae using streptomycete and non-streptomycete actinobacteria in the United Arab Emirates. *Frontiers in Microbiology*, *9*, 829.

Karami, P., Khaledi, A., Mashoof, R. Y., Yaghoobi, M. H., Karami, M., Dastan, D., & Alikhani, M. Y. (2020). The correlation between biofilm formation capability and antibiotic resistance pattern in Pseudomonas aeruginosa. *Gene Reports*, *18*, 100561.

Kassinger, S. J., & van Hoek, M. L. (2020). Biofilm architecture: An emerging synthetic biology target. *Synthetic and Systems Biotechnology*, *5*(1), 1–10.

Khatoon, Z., McTiernan, C. D., Suuronen, E. J., Mah, T. F., & Alarcon, E. I. (2018). *Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4: e01067*.

Kim, Y.-G., Lee, J.-H., Kim, C.-J., Lee, J.-C., Ju, Y. J., Cho, M. H., & Lee, J. (2012). Antibiofilm activity of Streptomyces sp. BFI 230 and Kribbella sp. BFI 1562 against Pseudomonas aeruginosa. *Applied Microbiology and Biotechnology*, *96*, 1607–1617.

Knobloch, J. K.-M., Horstkotte, M. A., Rohde, H., & Mack, D. (2002). Evaluation of different detection methods of biofilm formation in Staphylococcus aureus. *Medical Microbiology and Immunology*, *191*, 101–106.

Koley, S., & Mukherjee, M. (2021). Genetic Basis of Biofilm Formation and Spread of Nosocomial Infections. In *Analytical Methodologies for Biofilm Research* (pp. 269–298). Springer.

Kotoky, R., Rajkumari, J., & Pandey, P. (2018). The rhizosphere microbiome: Significance in rhizoremediation of polyaromatic hydrocarbon contaminated soil. *Journal of Environmental Management*, *217*, 858–870. https://doi.org/https://doi.org/10.1016/j.jenvman.2018.04.022

Kranjec, C., Morales Angeles, D., Torrissen Mårli, M., Fernández, L., García, P., Kjos, M., & Diep, D. B. (2021). Staphylococcal biofilms: Challenges and novel therapeutic perspectives. *Antibiotics*, *10*(2), 131.

Krysenko, S. (2024). *Synthetic Biology Approaches for Engineering of Secondary Metabolism in Streptomyces: Manipulation of Crosstalk between Primary and Secondary Metabolism*.

Krysenko, S., & Wohlleben, W. (2024). Role of Carbon, Nitrogen, Phosphate and Sulfur Metabolism in Secondary Metabolism Precursor Supply in Streptomyces spp. *Microorganisms*, *12*(8), 1571.

Kuncharoen, N., Pittayakhajonwut, P., & Tanasupawat, S. (2018). Micromonospora globbae sp. nov., an endophytic actinomycete isolated from roots of Globba winitii CH Wright. *International Journal of Systematic and Evolutionary Microbiology*, *68*(4), 1073–1077.

Lahiri, D., Nag, M., Dey, A., Sarkar, T., Ray, R. R., Rebezov, M., Shariati, M. A., Thiruvengadam, M., & Simal-Gandara, J. (2022). Immobilized enzymes as potent

antibiofilm agent. *Biotechnology Progress*, *38*(5), e3281.

Law, J. W.-F., Law, L. N.-S., Letchumanan, V., Tan, L. T.-H., Wong, S. H., Chan, K.-G., Ab Mutalib, N.-S., & Lee, L.-H. (2020). Anticancer Drug Discovery from Microbial Sources: The Unique Mangrove Streptomycetes. *Molecules (Basel, Switzerland)*, *25*(22). https://doi.org/10.3390/molecules25225365

Lawson, P. A. (2018). The phylum actinobacteria. In *The Bifidobacteria and Related Organisms* (pp. 1–8). Elsevier.

Lay Jr, J. O. (2001). MALDI-TOF mass spectrometry of bacteria. *Mass Spectrometry Reviews*, *20*(4), 172–194.

Leetanasaksakul, K., & Thamchaipenet, A. (2018). Potential anti-biofilm producing marine actinomycetes isolated from sea sediments in Thailand. *Agriculture and Natural Resources*, *52*(3), 228–233.

LewisOscar, F., Nithya, C., Vismaya, S., Arunkumar, M., Pugazhendhi, A., Nguyen-Tri, P., Alharbi, S. A., Alharbi, N. S., & Thajuddin, N. (2021). In vitro analysis of green fabricated silver nanoparticles (AgNPs) against Pseudomonas aeruginosa PA14 biofilm formation, their application on urinary catheter. *Progress in Organic Coatings*, *151*, 106058.

Li, T., He, L., Song, Y., Villaruz, A. E., Joo, H.-S., Liu, Q., Zhu, Y., Wang, Y., Qin, J., & Otto, M. (2016). AraC-type regulator Rsp adapts Staphylococcus aureus gene expression to acute infection. *Infection and Immunity*, *84*(3), 723–734.

Li, W.-J., Jiao, J., Salam, N., & Rao, M. P. N. (2024). *Modern Taxonomy of Bacteria and Archaea: New Methods, Technology and Advances*. Springer Nature.

Li, Y.-H., & Tian, X. (2012). Quorum sensing and bacterial social interactions in biofilms. *Sensors*, *12*(3), 2519–2538.

Lister, J. L., & Horswill, A. R. (2014). Staphylococcus aureus biofilms: recent developments in biofilm dispersal. *Frontiers in Cellular and Infection Microbiology*, *4*, 178.

Lob, S. H., DePestel, D. D., DeRyke, C. A., Kazmierczak, K. M., Young, K., Motyl, M. R., & Sahm, D. F. (2021). Ceftolozane/Tazobactam and Imipenem/Relebactam Cross-Susceptibility Among Clinical Isolates of Pseudomonas aeruginosa From Patients With Respiratory Tract Infections in ICU and Non-ICU Wards—SMART United States

2017–2019. *Open Forum Infectious Diseases*, *8*(7), ofab320.

Lopes, L. Q. S., Guerim, P., Santos, R. C. V., Marquezan, F. K., & Marquezan, P. K. (2023). The influence of different culture media on Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus biofilm formation. *Bioscience Journal*, *39*(e39096), 1981–3163.

Lv, Y., Liu, X., Zhou, S., Yu, Q., & Xu, Y. (2022). Microbial saccharification–biorefinery platform for lignocellulose. *Industrial Crops and Products*, *189*, 115761.

Maftei, N.-M., Raileanu, C. R., Balta, A. A., Ambrose, L., Boev, M., Marin, D. B., & Lisa, E. L. (2024). The Potential Impact of Probiotics on Human Health: An Update on Their Health-Promoting Properties. *Microorganisms*, *12*(2), 234.

Martegani, E., Bolognese, F., Trivellin, N., & Orlandi, V. T. (2020). Effect of blue light at 410 and 455 nm on Pseudomonas aeruginosa biofilm. *Journal of Photochemistry and Photobiology B: Biology*, *204*, 111790.

Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., Horner-Devine, M. C., Kane, M., Krumins, J. A., & Kuske, C. R. (2006). Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology*, *4*(2), 102–112.

Mary, T. R. J., Kannan, R. R., Iniyan, A. M., Ramachandran, D., & Vincent, S. G. P. (2021). Cell wall distraction and biofilm inhibition of marine Streptomyces derived angucycline in methicillin resistant Staphylococcus aureus. *Microbial Pathogenesis*, *150*, 104712.

Mathur, T., Singhal, S., Khan, S., Upadhyay, D. J., Fatma, T., & Rattan, A. (2006). Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. *Indian Journal of Medical Microbiology*, *24*(1), 25–29.

Meklat, A., Bouras, N., Mokrane, S., Zitouni, A., Djemouai, N., Klenk, H.-P., Sabaou, N., & Mathieu, F. (2020). Isolation, classification and antagonistic properties of alkalitolerant actinobacteria from Algerian Saharan soils. *Geomicrobiology Journal*, *37*(9), 826–836.

Merritt, J. H., Kadouri, D. E., & O'Toole, G. A. (2011). Growing and analyzing static biofilms. *Current Protocols in Microbiology*, *22*(1), 1B – 1.

Messaoudi, O., Wink, J., & Bendahou, M. (2020). Diversity of Actinobacteria Isolated from

Date Palms Rhizosphere and Saline Environments: Isolation, Identification and Biological Activity Evaluation. *Microorganisms*, *8*(12), 1853.

Michaelis, C., & Grohmann, E. (2023). Horizontal gene transfer of antibiotic resistance genes in biofilms. *Antibiotics*, *12*(2), 328.

Miethke, M., Pieroni, M., Weber, T., Brönstrup, M., Hammann, P., Halby, L., Arimondo, P. B., Glaser, P., Aigle, B., & Bode, H. B. (2021). Towards the sustainable discovery and development of new antibiotics. *Nature Reviews Chemistry*, *5*(10), 726–749.

Mika, F., & Hengge, R. (2013). Small regulatory RNAs in the control of motility and biofilm formation in E. coli and Salmonella. *International Journal of Molecular Sciences*, *14*(3), 4560–4579.

Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., & Huynh, T. (2022). Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiology*, *8*(3), 239–277.

Mishra, R., Panda, A. K., De Mandal, S., Shakeel, M., Bisht, S. S., & Khan, J. (2020). Natural anti-biofilm agents: strategies to control biofilm-forming pathogens. *Frontiers in Microbiology*, *11*, 566325.

Mohamed, H., Miloud, B., Zohra, F., García-Arenzana, J. M., Veloso, A., & Rodríguez-Couto, S. (2017). Isolation and characterization of actinobacteria from Algerian sahara soils with antimicrobial activities. *International Journal of Molecular and Cellular Medicine*, *6*(2), 109.

Moormeier, D. E., & Bayles, K. W. (2017). Staphylococcus aureus biofilm: a complex developmental organism. *Molecular Microbiology*, *104*(3), 365–376.

Muazi Alenazi, A., Anwar, Y., EM Abo-Aba, S., & M Bataweel, N. (2023). A review on Actinomycetes distribution, isolation, and their medical applications. *Novel Research in Microbiology Journal*, *7*(2), 1918–1931.

Neopane, P., Nepal, H. P., Shrestha, R., Uehara, O., & Abiko, Y. (2018). In vitro biofilm formation by Staphylococcus aureus isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. *International Journal of General Medicine*, *11*, 25.

Nguyen, H. T. T., Nguyen, T. H., & Otto, M. (2020). The staphylococcal exopolysaccharide

PIA–Biosynthesis and role in biofilm formation, colonization, and infection. *Computational and Structural Biotechnology Journal*, *18*, 3324–3334.

Nouioui, I., Carro, L., García-López, M., Meier-Kolthoff, J. P., Woyke, T., Kyrpides, N. C., Pukall, R., Klenk, H.-P., Goodfellow, M., & Göker, M. (2018). Genome-based taxonomic classification of the phylum Actinobacteria. *Frontiers in Microbiology*, *9*, 2007.

O'Toole, G., Kaplan, H. B., & Kolter, R. (2000). Biofilm formation as microbial development. In *Annual Review of Microbiology* (Vol. 54). https://doi.org/10.1146/annurev.micro.54.1.49

Obaid, K. A. (2019). Effect of Lactobacillus acidophilus Supernatant on the Biofilm Formation of Some Pathogenic Bacteria. *Journal of University of Babylon for Pure and Applied Sciences*, *27*(4), 72–90.

Olivares, E., Badel-Berchoux, S., Provot, C., Jaulhac, B., Prévost, G., Bernardi, T., & Jehl, F. (2016). The BioFilm Ring Test: a rapid method for routine analysis of Pseudomonas aeruginosa biofilm formation kinetics. *Journal of Clinical Microbiology*, *54*(3), 657–661.

Olivares, E., Tasse, J., Badel-Berchoux, S., Provot, C., Prévost, G., & Bernardi, T. (2020). Clinical biofilm ring test® reveals the potential role of β-lactams in the induction of biofilm formation by p. aeruginosa in cystic fibrosis patients. *Pathogens*, *9*(12), 1065.

Oskay, A. M., Üsame, T., & Cem, A. (2004). Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. *African Journal of Biotechnology*, *3*(9), 441–446.

Öztürk, F. Y., Darcan, C., & Kariptaş, E. (2023). The determination, monitoring, molecular mechanisms and formation of biofilm in E. coli. *Brazilian Journal of Microbiology*, *54*(1), 259–277.

Paleczny, J., Brożyna, M., Dudek-Wicher, R., Dydak, K., Oleksy-Wawrzyniak, M., Madziała, M., Bartoszewicz, M., & Junka, A. (2022). The Medium Composition Impacts Staphylococcus aureus Biofilm Formation and Susceptibility to Antibiotics Applied in the Treatment of Bone Infections. *International Journal of Molecular Sciences*, *23*(19), 11564.

Park, S. R., Tripathi, A., Wu, J., Schultz, P. J., Yim, I., McQuade, T. J., Yu, F., Arevang, C.-J., Mensah, A. Y., Tamayo-Castillo, G., Xi, C., & Sherman, D. H. (2016). Discovery of cahuitamycins as biofilm inhibitors derived from a convergent biosynthetic pathway. *Nature Communications*, *7*(1), 10710. https://doi.org/10.1038/ncomms10710

Peng, Q., Tang, X., Dong, W., Sun, N., & Yuan, W. (2022). A review of biofilm formation of Staphylococcus aureus and its regulation mechanism. *Antibiotics*, *12*(1), 12.

Peñil Cobo, M., Libro, S., Marechal, N., D'Entremont, D., Peñil Cobo, D., & Berkmen, M. (2018). Visualizing Bacterial Colony Morphologies Using Time-Lapse Imaging Chamber MOCHA. In T. J. Silhavy (Ed.), *Journal of Bacteriology* (Vol. 200, Issue 2). https://doi.org/10.1128/JB.00413-17

Perry, E. K., & Tan, M.-W. (2023). Bacterial biofilms in the human body: Prevalence and impacts on health and disease. *Frontiers in Cellular and Infection Microbiology*, *13*, 1237164.

Picoli, T., Peter, C. M., Zani, J. L., Waller, S. B., Lopes, M. G., Boesche, K. N., de Oliveira Hübner, S., & Fischer, G. (2017). Melittin and its potential in the destruction and inhibition of the biofilm formation by Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa isolated from bovine milk. *Microbial Pathogenesis*, *112*, 57–62.

Pinto, R. M., Lopes-de-Campos, D., Martins, M. C. L., Van Dijck, P., Nunes, C., & Reis, S. (2019). Impact of nanosystems in Staphylococcus aureus biofilms treatment. *FEMS Microbiology Reviews*, *43*(6), 622–641.

Pinto, R. M., Seabra, C. L., De Jonge, M., Martins, M. C. L., Van Dijck, P., Reis, S., & Nunes, C. (2022). Antibiofilm combinatory strategy: moxifloxacin-loaded nanosystems and encapsulated N-Acetyl-L-Cysteine. *Pharmaceutics*, *14*(11), 2294.

Pommerville, J. C. (2013). *Alcamo's Fundamentals of Microbiology: Body systems edition*. Jones & Bartlett Publishers.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health*, *109*(7), 309–318.

Prudence, S. M. M., Addington, E., Castaño-Espriu, L., Mark, D. R., Pintor-Escobar, L., Russell, A. H., & McLean, T. C. (2020). Advances in actinomycete research: an

ActinoBase review of 2019. *Microbiology (Reading, England)*, *166*(8), 683–694. https://doi.org/10.1099/mic.0.000944

Pugazhendhi, A., Michael, D., Prakash, D., Krishnamaurthy, P. P., Shanmuganathan, R., Al-Dhabi, N. A., Duraipandiyan, V., Arasu, M. V., & Kaliannan, T. (2020). Antibiogram and plasmid profiling of beta-lactamase producing multi drug resistant Staphylococcus aureus isolated from poultry litter. *Journal of King Saud University-Science*, *32*(6), 2723–2727.

Quinn, G. A., Banat, A. M., Abdelhameed, A. M., & Banat, I. M. (2020). Streptomyces from traditional medicine: sources of new innovations in antibiotic discovery. *Journal of Medical Microbiology*, *69*(8), 1040–1048. https://doi.org/10.1099/jmm.0.001232

Radaic, A., & Kapila, Y. L. (2021). The oralome and its dysbiosis: New insights into oral microbiome-host interactions. *Computational and Structural Biotechnology Journal*, *19*, 1335–1360.

Rajivgandhi, G., Vijayan, R., Maruthupandy, M., Vaseeharan, B., & Manoharan, N. (2018). Antibiofilm effect of Nocardiopsis sp. GRG 1 (KT235640) compound against biofilm forming Gram negative bacteria on UTIs. *Microbial Pathogenesis*, *118*, 190–198.

Rajoka, M. I. (1999). *Cellulomonas*.

Rammali, S., Rahim, A., El Aalaoui, M., Bencharki, B., Dari, K., Habach, A., Abdeslam, L., & Khattabi, A. (2024). Antimicrobial potential of Streptomyces coeruleofuscus SCJ isolated from microbiologically unexplored garden soil in Northwest Morocco. *Scientific Reports*, *14*(1), 3359.

Rasamiravaka, T., Labtani, Q., Duez, P., & El Jaziri, M. (2015). The formation of biofilms by Pseudomonas aeruginosa: a review of the natural and synthetic compounds interfering with control mechanisms. *BioMed Research International*, *2015*.

Razdan, K., Garcia-Lara, J., Sinha, V. R., & Singh, K. K. (2022). Pharmaceutical strategies for the treatment of bacterial biofilms in chronic wounds. *Drug Discovery Today*, *27*(8), 2137–2150.

Reffuveille, F., Josse, J., Vallé, Q., Gangloff, C. M., & Gangloff, S. C. (2017). Staphylococcus aureus Biofilms and their Impact on the Medical Field. *The Rise of Virulence and Antibiotic Resistance in Staphylococcus Aureus*, *11*, 187.

REFERENCES

Reggani, K., Drici, H., & Ghallai, L. (2021). Morphological characterization and preliminary screening for antibacterial activity of Actinobacteria strains isolated from Tamanrasset soil as a hyper arid region in southern Algeria. *Algerian Journal of Arid Environment "AJAE," 11*(2), 18.

Rendueles, O., Kaplan, J. B., & Ghigo, J. (2013). Antibiofilm polysaccharides. *Environmental Microbiology*, *15*(2), 334–346.

Roy, B., Maitra, D., & Mitra, A. K. (2021). Methods of sample preparation and assay of bacterial biofilms with special reference to their significance in agriculture and extreme environments. *Analytical Methodologies for Biofilm Research*, 39–65.

Rumbaugh, K. P., & Sauer, K. (2020). Biofilm dispersion. *Nature Reviews Microbiology*, *18*(10), 571–586.

Rychert, J. (2019). Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. *Journal of Infectiology and Epidemiology*, *2*(4).

Sahle, Z., Engidaye, G., Shenkute, D., Metaferia, Y., & Shibabaw, A. (2022). High Prevalence of Multi-Drug Resistance and Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae Among Hospitalized Patients Presumptive for Bacterial Infection at Debre Berhan Comprehensive Specialized Hospital, Ethiopia. *Infection and Drug Resistance*, 2639–2656.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425.

Salam, M. A., Al-Amin, M. Y., Pawar, J. S., Akhter, N., & Lucy, I. B. (2023). Conventional methods and future trends in antimicrobial susceptibility testing. *Saudi Journal of Biological Sciences*, *30*(3), 103582.

Salam, N., Jiao, J.-Y., Zhang, X.-T., & Li, W.-J. (2020). Update on the classification of higher ranks in the phylum Actinobacteria. *International Journal of Systematic and Evolutionary Microbiology*, *70*(2), 1331–1355.

Saleem, H. G. M., Aftab, U., Sajid, I., Abbas, Z., & Sabri, A. N. (2015). Effect of crude extracts of selected actinomycetes on biofilm formation of A. schindleri, M. aci, and B. cereus. *Journal of Basic Microbiology*, *55*(5), 645–651.

Sapkota, A., Thapa, A., Budhathoki, A., Sainju, M., Shrestha, P., & Aryal, S. (2020).

Isolation, characterization, and screening of antimicrobial-producing actinomycetes from soil samples. *International Journal of Microbiology, 2020*.

Sauer, K., Stoodley, P., Goeres, D. M., Hall-Stoodley, L., Burmølle, M., Stewart, P. S., & Bjarnsholt, T. (2022). The biofilm life cycle: Expanding the conceptual model of biofilm formation. *Nature Reviews Microbiology*, *20*(10), 608–620.

Sayem, S. M. A., Manzo, E., Ciavatta, L., Tramice, A., Cordone, A., Zanfardino, A., De Felice, M., & Varcamonti, M. (2011). Anti-biofilm activity of an exopolysaccharide from a sponge-associated strain of Bacillus licheniformis. *Microbial Cell Factories, 10*, 1–12.

Schulze, A., Mitterer, F., Pombo, J. P., & Schild, S. (2021). Biofilms by bacterial human pathogens: Clinical relevance-development, composition and regulation-therapeutical strategies. *Microbial Cell*, *8*(2), 28.

Shaaban, M., Elgaml, A., & Habib, E.-S. E. (2019). Biotechnological applications of quorum sensing inhibition as novel therapeutic strategies for multidrug resistant pathogens. *Microbial Pathogenesis*, *127*, 138–143.

Sharma, M., Dangi, P., & Choudhary, M. (2014). *Actinomycetes : Source , Identification , and Their Applications*. *3*(2), 801–832.

Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkeel, R. (2023). Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*, *11*(6), 1614.

Shirling, E. B. T., & Gottlieb, D. (1966). Methods for characterization of Streptomyces species. *International Journal of Systematic Bacteriology*, *16*(3), 313–340.

Shree, P., Singh, C. K., Sodhi, K. K., Surya, J. N., & Singh, D. K. (2023). Biofilms: Understanding the structure and contribution towards bacterial resistance in antibiotics. *Medicine in Microecology*, *16*, 100084.

Shrestha, L., Fan, H.-M., Tao, H.-R., & Huang, J.-D. (2022). Recent strategies to combat biofilms using antimicrobial agents and therapeutic approaches. *Pathogens*, *11*(3), 292.

Silva, E., Teixeira, J. A., Pereira, M. O., Rocha, C. M. R., & Sousa, A. M. (2023). Evolving biofilm inhibition and eradication in clinical settings through plant-based antibiofilm agents. *Phytomedicine*, 154973.

REFERENCES

Singh, A. K., Prakash, P., Achra, A., Singh, G. P., Das, A., & Singh, R. K. (2017). Standardization and classification of in vitro biofilm formation by clinical isolates of Staphylococcus aureus. *Journal of Global Infectious Diseases*, *9*(3), 93.

Singh, R., & Dubey, A. K. (2020). Isolation and characterization of a new endophytic actinobacterium Streptomyces californicus strain ADR1 as a promising source of anti-bacterial, anti-biofilm and antioxidant metabolites. *Microorganisms*, *8*(6), 929.

Singh, S., Datta, S., Narayanan, K. B., & Rajnish, K. N. (2021). Bacterial exo-polysaccharides in biofilms: role in antimicrobial resistance and treatments. *Journal of Genetic Engineering and Biotechnology*, *19*, 1–19.

Sionov, R. V., & Steinberg, D. (2022). Targeting the holy triangle of quorum sensing, biofilm formation, and antibiotic resistance in pathogenic bacteria. *Microorganisms*, *10*(6), 1239.

Sreevidya, M., Gopalakrishnan, S., Kudapa, H., & Varshney, R. K. (2016). Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. *Brazilian Journal of Microbiology*, *47*(1), 85–95. https://doi.org/10.1016/j.bjm.2015.11.030

Stevens, E. J., Bates, K. A., & King, K. C. (2021). Host microbiota can facilitate pathogen infection. *PLoS Pathogens*, *17*(5), e1009514.

Suzuki, N., Ohtaguro, N., Yoshida, Y., Hirai, M., Matsuo, H., Yamada, Y., Imamura, N., & Tsuchiya, T. (2015). A compound inhibits biofilm formation of Staphylococcus aureus from Streptomyces. *Biological and Pharmaceutical Bulletin*, *38*(6), 889–892.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, *101*(30), 11030–11035.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027.

Thenmozhi, R., Nithyanand, P., Rathna, J., & Karutha Pandian, S. (2009). Antibiofilm activity of coral-associated bacteria against different clinical M serotypes of Streptococcus pyogenes. *FEMS Immunology & Medical Microbiology*, *57*(3), 284–294.

Thi, M. T. T., Wibowo, D., & Rehm, B. H. A. (2020). Pseudomonas aeruginosa biofilms.

International Journal of Molecular Sciences, *21*(22), 8671.

Torday, J., Miller Jr, W., Torday, J., & Miller Jr, W. (2020). Holobionts. *Cellular-Molecular Mechanisms in Epigenetic Evolutionary Biology*, 93–101.

Tuon, F. F., Suss, P. H., Telles, J. P., Dantas, L. R., Borges, N. H., & Ribeiro, V. S. T. (2023). Antimicrobial treatment of Staphylococcus aureus biofilms. *Antibiotics*, *12*(1), 87.

Urban-Chmiel, R., Marek, A., Stępień-Pyśniak, D., Wieczorek, K., Dec, M., Nowaczek, A., & Osek, J. (2022). Antibiotic resistance in bacteria—A review. *Antibiotics*, *11*(8), 1079.

Usui, M., Yoshii, Y., Thiriet-Rupert, S., Ghigo, J.-M., & Beloin, C. (2023). Intermittent antibiotic treatment of bacterial biofilms favors the rapid evolution of resistance. *Communications Biology*, *6*(1), 275.

Vashistha, A., Sharma, N., Nanaji, Y., Kumar, D., Singh, G., Barnwal, R. P., & Yadav, A. K. (2023). Quorum sensing inhibitors as Therapeutics: Bacterial biofilm inhibition. *Bioorganic Chemistry*, *136*, 106551.

Vazquez, N. M., Mariani, F., Torres, P. S., Moreno, S., & Galvan, E. M. (2020). Cell death and biomass reduction in biofilms of multidrug resistant extended spectrum β-lactamase-producing uropathogenic Escherichia coli isolates by 1, 8-cineole. *PloS One*, *15*(11), e0241978.

Velho-Pereira, S., & Kamat, N. M. (2011). Antimicrobial screening of actinobacteria using a modified cross-streak method. *Indian Journal of Pharmaceutical Sciences*, *73*(2), 223.

Vestby, L. K., Grønseth, T., Simm, R., & Nesse, L. L. (2020). Bacterial biofilm and its role in the pathogenesis of disease. *Antibiotics*, *9*(2), 59.

Vishnupriya, C., Mohamedrizwan, K., Arya, P. R., Vijayakumar, S., & Kavitha, R. (2024). Evaluation of heavy metal removal and antibiofilm efficiency of biologically synthesized chitosan-silver nano-bio composite by a soil actinobacterium Glutamicibacter uratoxydans VRAK 24. *International Journal of Biological Macromolecules*, *255*, 128032.

Vurukonda, S. S. K. P., Giovanardi, D., & Stefani, E. (2018). Plant growth promoting and biocontrol activity of Streptomyces spp. as endophytes. *International Journal of Molecular Sciences*, *19*(4), 952.

Wang, A., Zhang, Y., Wang, G., & Zhang, Z. (2024). Soil physicochemical properties and microorganisms jointly regulate the variations of soil carbon and nitrogen cycles along vegetation restoration on the Loess Plateau, China. *Plant and Soil*, *494*(1), 413–436.

Waturangi, D. E., Rahayu, B. S., Kezia Yolanda Lalu, M., & Mulyono, N. (2016). Characterization of bioactive compound from actinomycetes for antibiofilm activity against gram-negative and gram-positive bacteria. *Malaysian Journal of Microbiology*, 291–299.

Wimpenny, J., Manz, W., & Szewzyk, U. (2000). Heterogeneity in biofilms. *FEMS Microbiology Reviews*, *24*(5), 661–671.

Winans, J. B., Wucher, B. R., & Nadell, C. D. (2022). Multispecies biofilm architecture determines bacterial exposure to phages. *PLoS Biology*, *20*(12), e3001913.

Wu, X., Wang, H., Xiong, J., Yang, G.-X., Hu, J.-F., Zhu, Q., & Chen, Z. (2024). Staphylococcus aureus biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. *Biofilm*, 100175.

Xie, F., & Pathom-Aree, W. (2021). Actinobacteria from desert: diversity and biotechnological applications. *Frontiers in Microbiology*, *12*, 765531.

Xu, L., Li, Q., & Jiang, C. (1996). Diversity of soil actinomycetes in Yunnan, China. *Applied and Environmental Microbiology*, *62*(1), 244–248.

Yadav, A. N., Verma, P., Kumar, S., Kumar, V., Kumar, M., Sugitha, T. C. K., Singh, B. P., Saxena, A. K., & Dhaliwal, H. S. (2018). Actinobacteria from Rhizosphere: Molecular Diversity, Distributions, and Potential Biotechnological Applications. In B. P. Singh, V. K. Gupta, & A. K. Passari (Eds.), *New and Future Developments in Microbial Biotechnology and Bioengineering* (pp. 13–41). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-444-63994-3.00002-3

Yadav, J., Kumari, R. M., Verma, V., & Nimesh, S. (2021). Recent development in therapeutic strategies targeting Pseudomonas aeruginosa biofilms–A review. *Materials Today: Proceedings*, *46*, 2359–2373.

Yan, C.-H., Chen, F.-H., Yang, Y.-L., Zhan, Y.-F., Herman, R. A., Gong, L.-C., Sheng, S., & Wang, J. (2023). The transcription factor CsgD contributes to engineered Escherichia coli resistance by regulating biofilm formation and stress responses. *International*

Journal of Molecular Sciences, *24*(18), 13681.

Yin, R., Cheng, J., Wang, J., Li, P., & Lin, J. (2022). Treatment of Pseudomonas aeruginosa infectious biofilms: challenges and strategies. *Frontiers in Microbiology*, 3325.

You, J., Xue, X., Cao, L., Lu, X., Wang, J., Zhang, L., & Zhou, S. (2007). Inhibition of Vibrio biofilm formation by a marine actinomycete strain A66. *Applied Microbiology and Biotechnology*, *76*, 1137–1144.

Yuceel-Timur, I., Garner, C. D., Franklin, S., & Hardy, D. J. (2024). Performance evaluation of cefoxitin screen test on two different automated antimicrobial susceptibility test systems: a comparative study. *Microbiology Spectrum*, *12*(9), e03815-23.

Zacchetti, B., Andrianos, A., van Dissel, D., de Ruiter, E., van Wezel, G. P., & Claessen, D. (2018). Microencapsulation extends mycelial viability of Streptomyces lividans 66 and increases enzyme production. *BMC Biotechnology*, *18*(1), 13. https://doi.org/10.1186/s12896-018-0425-2

Zamoum, M., Goudjal, Y., Sabaou, N., Barakate, M., Mathieu, F., & Zitouni, A. (2015). Biocontrol capacities and plant growth-promoting traits of endophytic actinobacteria isolated from native plants of Algerian Sahara. *Journal of Plant Diseases and Protection*, *122*(5–6), 215–223.

Zhang, Q.-Y., Yan, Z.-B., Meng, Y.-M., Hong, X.-Y., Shao, G., Ma, J.-J., Cheng, X.-R., Liu, J., Kang, J., & Fu, C.-Y. (2021). Antimicrobial peptides: mechanism of action, activity and clinical potential. *Military Medical Research*, *8*, 1–25.

Zhao, A., Sun, J., & Liu, Y. (2023). Understanding bacterial biofilms: From definition to treatment strategies. *Frontiers in Cellular and Infection Microbiology*, *13*.

Zhou, F., Wang, D., Hu, J., Zhang, Y., Tan, B. K., & Lin, S. (2022). Control measurements of Escherichia coli biofilm: A review. *Foods*, *11*(16), 2469.

Zobell, C. E. (1943). The effect of solid surfaces upon bacterial activity. *Journal of Bacteriology*, *46*(1), 39–56.

Zou, M., & Liu, D. (2018). A systematic characterization of the distribution, biofilm-forming potential and the resistance of the biofilms to the CIP processes of the bacteria in a milk powder processing factory. *Food Research International*, *113*, 316–326.

REFERENCES

ANNEXES

ANNEX 1: Materials and products

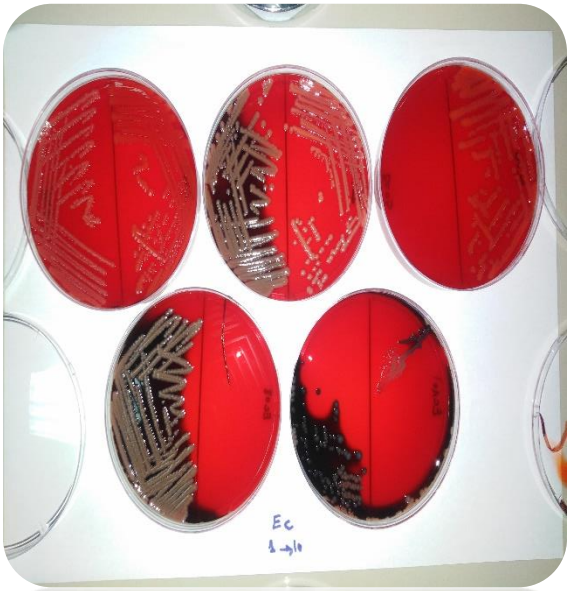
Equipment	
Heating rotary plate	
Balance	
Enzyme-linked immunosorbent assay reader	
Incubators, rotary incubator	
Light microscopy	
Rotary evaporator equipment	
Spectrophotometry	
Material	
Laboratory glassware and other needs (96 well–flat bottom polystyrene, Petri dishes, Whatman paper N° 1, micropipettes…etc.)	

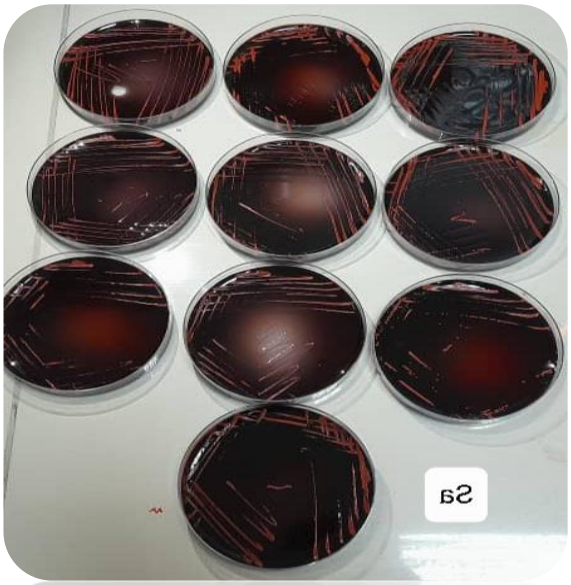
Products		
Nam	Brand	Expiration date
Culture mediums and tampons broths		
Chapman stone Agar	HIMEDIA; ref: M212-500G	2025
Hektoen Enteric Agar	HIMEDIA; ref: M467-500G	2025
HiCrom™ E coli Agar	HIMEDIA; ref:M12951-500G	2025
Brain heart infusion broth	BIOCHEM CHEMOPHARMA; ref: DM2820500, 500G	2025
Yeast extract	CONDALAB ; réf.: 1702, 500G	2025
Chemicals, Organic, inorganic products and organic solvents		
Acetate Ethyl	BIOCHEM CHEMOPHARMA, ref: 205102500-4	2027
Bacteriological Agar	CONDALAB; ref: 1803, 500G	2025
Congo red stain	SIGMA-ALDRICH ref: C6277-25g	2023
Fuchsine	SIGMA-ALDRICH ref: 87794-250ML	2025

ANNEXES

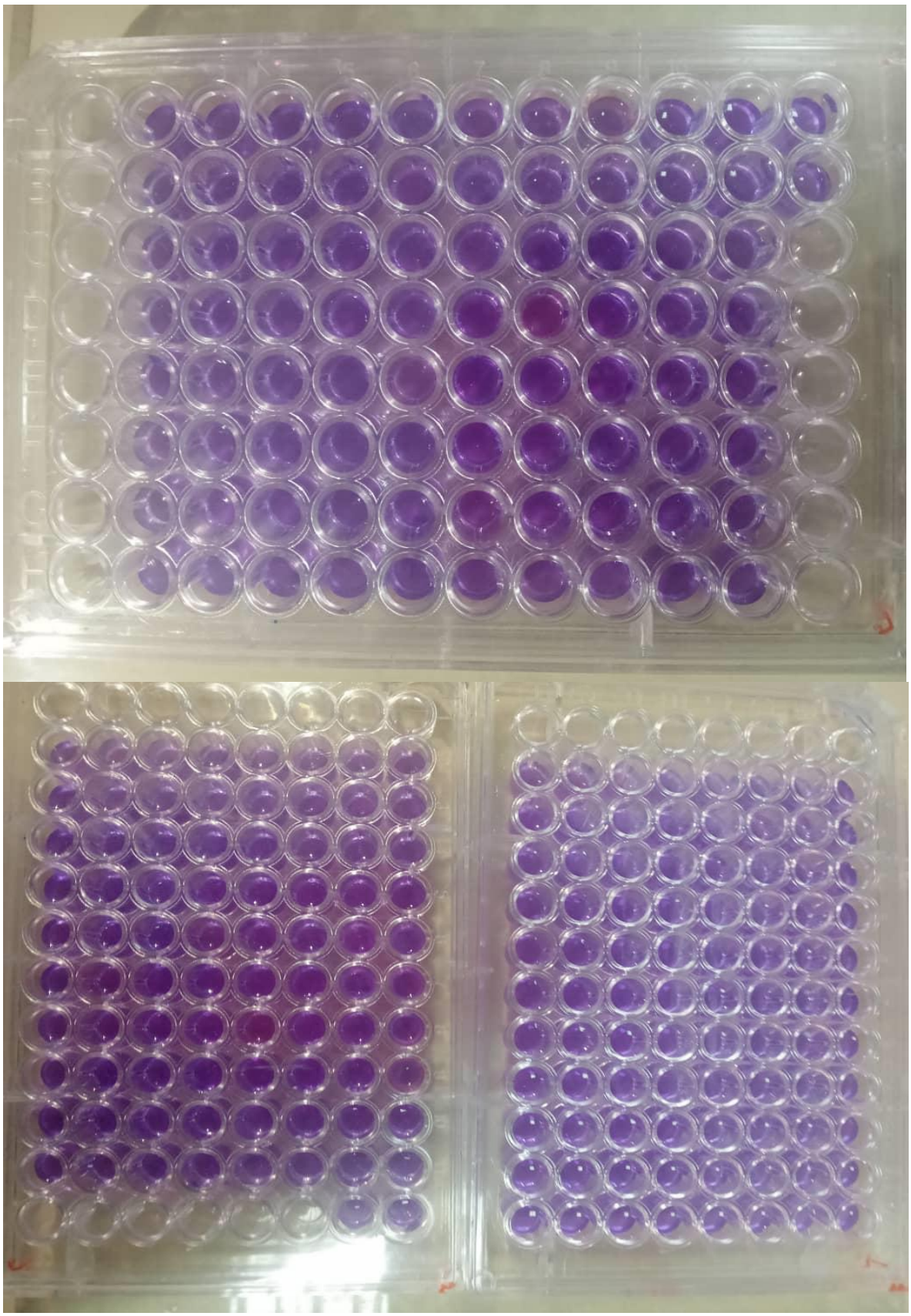
Cristal Violet	SIGMA-ALDRICH ref: 944448-250Ml	2025
Ethanol	SIGMA ALDRICH ref: 1009741011	2027
Glycerol	SPECILAB, ref: SP000953	2027
K_2HPO_4	BIOCHEM CHEMOPHARMA; ref: 304110500	2025
Methanol	BIOCHEM CHEMOPHARMA; ref:2130322500	2025
NaCl	BIOCHEM CHEMOPHARMA,; ref: 319120500	2027
Sodium acetate	BIOCHEM CHEMOPHARMA; ref: 319770500	2025
Sucrose	BIOCHEM CHEMOPHARMA; ref:	2025

ANNEX 2: results of CRO, TAM, MTP methods.



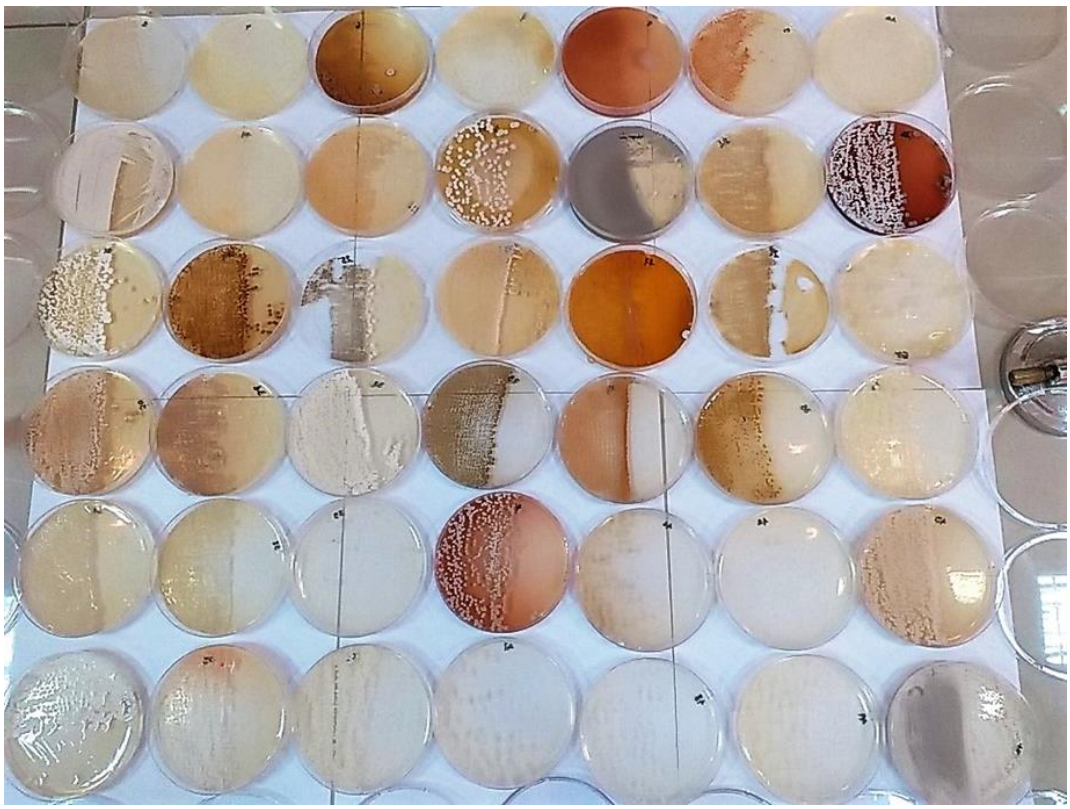


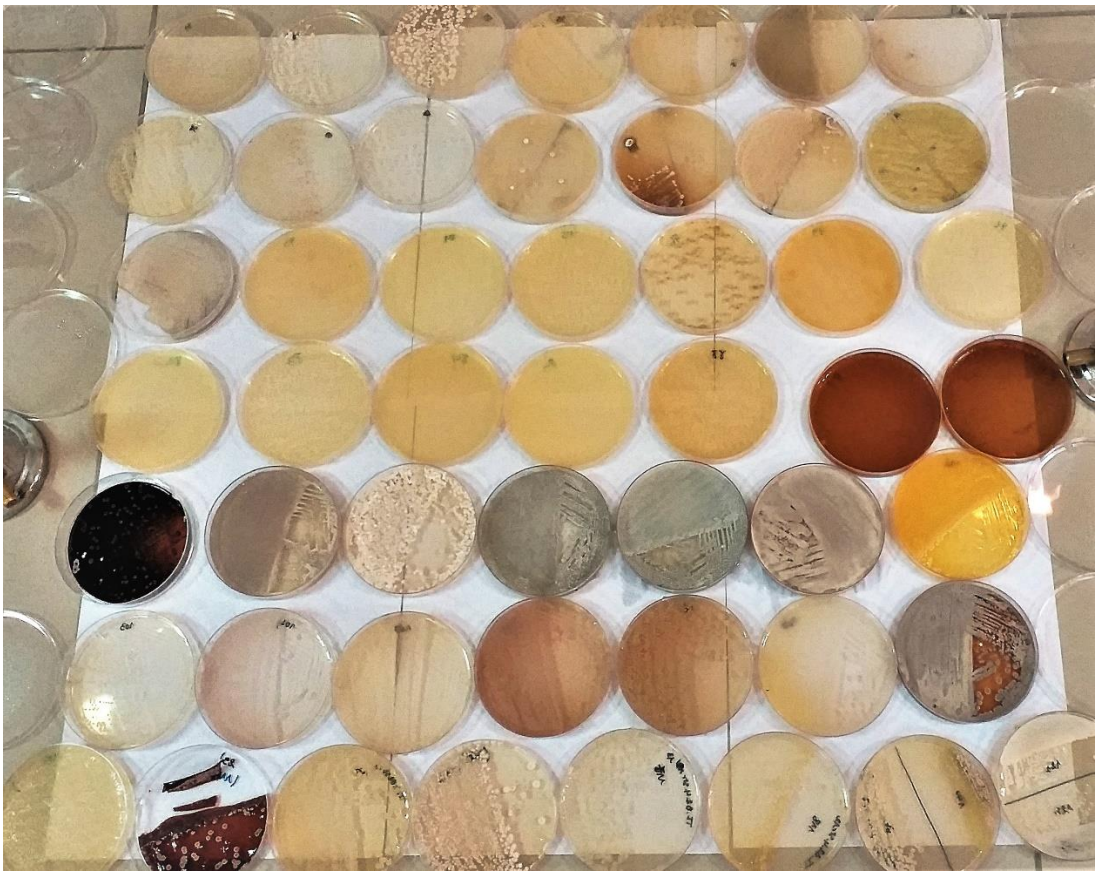
ANNEXES



ANNEXES

ANNEX 3: results of Actinobacteria isolation, macroscopic and microscopic consistency. And cross streak assays





ANNEXES

ANNEX 4: Antibiogramme of the clinical strains.

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

Page 1/2
22/12/2022 09:19:07

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF01**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:36
Receipt Date:20/12/2022 12:04:36
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☑	1	Complete	22/12/2022 01:47:06
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:04:37

Organism Name		Comments	Classification
1 ESCCOL	Escherichia coli		Significant / Unknown

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	R
Amoxicillin-Clavulanate (f)	32/2	R
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	>4/8	
Cefazolin	<=4	X
Cefepime	<=1	S
Cefixime	<=0.5	S
Cefotaxime	<=1	S
Ceftazidime	<=1	S
Ceftazidime-Avibactam	<=0.25/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	<=1	S
Cefuroxime	16	I
Ciprofloxacin	>1	R
Colistin	<=1	X

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

22/12/2022 09:19:07

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF01**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:36
Receipt Date:20/12/2022 12:04:36
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	<=0.25	S
Levofloxacin	>8	R
Meropenem	<=0.125	S
Ofloxacin	>2	X
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	2	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	>8/152	R

Signature: _____

ANNEXES

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

22/12/2022 09:16:14

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF02**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:41
Receipt Date:20/12/2022 12:04:41
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☑	1	Complete	22/12/2022 01:27:37
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:04:42

Organism Name		Comments	Classification
1 ESCCOL	Escherichia coli		Significant / Unknown

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	<=4	
Amoxicillin-Clavulanate (f)	<=2/2	S
Ampicillin	<=4	S
Ampicillin-Sulbactam (f)	<=1/8	
Cefazolin	<=4	X
Cefepime	<=1	S
Cefixime	<=0.5	S
Cefotaxime	<=1	S
Ceftazidime	<=1	S
Ceftazidime-Avibactam	<=0.25/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	<=1	S
Cefuroxime	<=4	S
Ciprofloxacin	0.25	S
Colistin	<=1	X

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

22/12/2022 09:16:14

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF02**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:41
Receipt Date:20/12/2022 12:04:41
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	<=0.25	S
Levofloxacin	<=0.5	S
Meropenem	<=0.125	S
Ofloxacin	1	S
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	<=1	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	<=1/19	S

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

Page 1/2
27/12/2022 10:41:24

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF03**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:47 Receipt Date:20/12/2022 12:04:47
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☐	1	Complete	24/12/2022 01:47:02
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:04:48

Organism Name		Comments	Classification
1 ESCCOL	Escherichia coli		Significant / Unknown

E. coli

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	
Amoxicillin-Clavulanate (f)	8/2	S
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	4/8	
Cefazolin	<=4	X
Cefepime	<=1	S
Cefixime	<=0.5	S
Cefotaxime	<=1	S
Ceftazidime	<=1	S
Ceftazidime-Avibactam	<=0.25/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	<=1	S
Cefuroxime	<=4	S
Ciprofloxacin	0.25	S
Colistin	<=1	X

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:41:24

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF03**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:47
Receipt Date:20/12/2022 12:04:47
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	<=0.25	S
Levofloxacin	<=0.5	S
Meropenem	<=0.125	S
Ofloxacin	1	S
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	<=1	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	>8/152	R

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:41:49

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUM04**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:08
Receipt Date:20/12/2022 12:05:08
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☐	1	Complete	24/12/2022 02:26:55
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:05:09

Organism Name
1 ESCCOL Escherichia coli

Comments

Classification
Significant / Unknown

E. coli

Resistance Markers
1 CARBD Class D Carbapenemase Producer

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	R
Amoxicillin-Clavulanate (f)	>32/2	R
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	>4/8	
Cefazolin	16	X
Cefepime	<=1	S
Cefixime	<=0.5	S
Cefotaxime	<=1	S
Ceftazidime	<=1	S
Ceftazidime-Avibactam	<=0.25/4	S
Ceftolozane-Tazobactam	2/4	S
Ceftriaxone	<=1	S
Cefuroxime	8	S

Signature: _____

X

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:41:49

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUM04**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:08 Receipt Date:20/12/2022 12:05:08
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ciprofloxacin	0.5	I
Colistin	<=1	X
Ertapenem	1	I
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	0.5	S
Levofloxacin	1	I
Meropenem	0.25	S
Ofloxacin	2	S
Piperacillin-Tazobactam	>32/4	X
Tigecycline	<=1	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	>8/152	R

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

Page 1/2
27/12/2022 10:40:35

Patient Name:
Birth Date:
Ordering Physician: Unspecified
User Name: Bhavya

Patient ID:
Patient Sex:

Accession #: **EcUM06**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:13
Receipt Date:20/12/2022 12:05:13
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☐	1	Complete	24/12/2022 00:27:11
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:05:14

Organism Name		Comments	Classification
1 ESCCOL	Escherichia coli		Significant / Unknown

E. coli

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	R
Amoxicillin-Clavulanate (f)	32/2	R
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	>4/8	
Cefazolin	<=4	X
Cefepime	<=1	S
Cefixime	<=0.5	S
Cefotaxime	<=1	S
Ceftazidime	<=1	S
Ceftazidime-Avibactam	0.5/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	<=1	S
Cefuroxime	8	S
Ciprofloxacin	>1	R
Colistin	<=1	X

Signature: _____

X

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:40:35

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUM06**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:13 Receipt Date:20/12/2022 12:05:13
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	<=0.25	S
Levofloxacin	>8	R
Meropenem	<=0.125	S
Ofloxacin	>2	X
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	<=1	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	>8/152	R

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:41:00

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUM07**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:19 Receipt Date:20/12/2022 12:05:19
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☐	1	Complete	24/12/2022 00:46:53
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:05:20

Organism Name		Comments	Classification
1 ESCCOL	Escherichia coli		Significant / Unknown

E.coli

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	
Amoxicillin-Clavulanate (f)	16/2	I
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	4/8	
Cefazolin	<=4	X
Cefepime	<=1	S
Cefixime	<=0.5	S
Cefotaxime	<=1	S
Ceftazidime	<=1	S
Ceftazidime-Avibactam	<=0.25/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	<=1	S
Cefuroxime	<=4	S
Ciprofloxacin	<=0.125	S
Colistin	<=1	X

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:41:00

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUM07**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:19
Receipt Date:20/12/2022 12:05:19
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	<=0.25	S
Levofloxacin	<=0.5	S
Meropenem	<=0.125	S
Ofloxacin	<=0.5	S
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	<=1	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	>8/152	R

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

Page 1/2
27/12/2022 10:40:13

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF08**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:57
Receipt Date:20/12/2022 12:04:57
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☐	1	Complete	24/12/2022 00:47:07
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:04:59

Organism Name		Comments	Classification
1 ESCCOL	Escherichia coli		Significant / Unknown

E. coli

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	
Amoxicillin-Clavulanate (f)	16/2	I
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	4/8	
Cefazolin	<=4	X
Cefepime	<=1	S
Cefixime	<=0.5	S
Cefotaxime	<=1	S
Ceftazidime	<=1	S
Ceftazidime-Avibactam	<=0.25/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	<=1	S
Cefuroxime	<=4	S
Ciprofloxacin	>1	R
Colistin	<=1	X

Signature: _____

X

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:40:13

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF08**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:57 Receipt Date:20/12/2022 12:04:57
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	<=0.25	S
Levofloxacin	>8	R
Meropenem	<=0.125	S
Ofloxacin	>2	X
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	<=1	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	<=1/19	S

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:40:13

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF08**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:04:57 Receipt Date:20/12/2022 12:04:57
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	<=0.25	S
Levofloxacin	>8	R
Meropenem	<=0.125	S
Ofloxacin	>2	X
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	<=1	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	<=1/19	S

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:39:46

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: EcUM09
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:24
Receipt Date:20/12/2022 12:05:24
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☐	1	Complete	23/12/2022 23:46:48
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:05:25

Organism Name
1 ESCCOL Escherichia coli

Comments
MDR (+)

Classification
Significant / Unknown
E. coli

Resistance Markers
1 ESBL Extended Spectrum Beta-lactamase

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	8	S
Amoxicillin	>32	R
Amoxicillin-Clavulanate (f)	>32/2	R
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	>4/8	
Cefazolin	>32	R
Cefepime	16	R (1)
Cefixime	>2	R
Cefotaxime	>4	R
Ceftazidime	>16	R
Ceftazidime-Avibactam	1/4	S
Ceftolozane-Tazobactam	1/4	S
Ceftriaxone	>4	R (1)
Cefuroxime	>16	R

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

Page 2/2
27/12/2022 10:39:46

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUM09**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:24
Receipt Date:20/12/2022 12:05:24
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ciprofloxacin	>1	R
Colistin	<=1	X
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	>4	X
Imipenem	<=0.25	S
Levofloxacin	>8	R
Meropenem	<=0.125	S
Ofloxacin	>2	X
Piperacillin-Tazobactam	8/4	S
Tigecycline	<=1	S
Tobramycin	>8	R
Trimethoprim-Sulfamethoxazole	>8/152	R

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:39:20

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF10**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:03
Receipt Date:20/12/2022 12:05:03
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☐	1	Complete	24/12/2022 00:47:01
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:05:04

Organism Name		Comments	Classification
1 ESCCOL	Escherichia coli		Significant / Unknown

E. coli

Drug	ESCCOL MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	R
Amoxicillin-Clavulanate (f)	32/2	R
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	>4/8	
Cefazolin	<=4	X
Cefepime	<=1	S
Cefixime	<=0.5	S
Cefotaxime	<=1	S
Ceftazidime	<=1	S
Ceftazidime-Avibactam	<=0.25/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	<=1	S
Cefuroxime	8	S
Ciprofloxacin	>1	R
Colistin	<=1	X

Signature: _____

ANNEXES

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:39:20

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **EcUF10**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:05:03 Receipt Date:20/12/2022 12:05:03
Antimicrobial Therapy:
Auftrag:

Drug	ESCCOL MIC/*Conc*	SIR
Ertapenem	<=0.25	S
Fosfomycin w/G6P	<=16	X
Gentamicin	<=1	S
Imipenem	<=0.25	S
Levofloxacin	>8	R
Meropenem	<=0.125	S
Ofloxacin	>2	X
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	<=1	S
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	>8/152	R

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

Page 1/2
22/12/2022 09:17:45

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **PaPM01**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:06:24 Receipt Date:20/12/2022 12:06:24
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☑	1	Complete	22/12/2022 04:06:52
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:06:25

Organism Name		Comments	Classification
1 PSEAER	Pseudomonas aeruginosa		Significant / Unknown

Drug	PSEAER MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	R
Amoxicillin-Clavulanate (f)	>32/2	R
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	>4/8	R
Cefazolin	>32	R
Cefepime	4	S
Cefixime	>2	
Cefotaxime	>4	R
Ceftazidime	>16	R
Ceftazidime-Avibactam	2/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	>4	R
Cefuroxime	>16	R
Ciprofloxacin	0.5	S
Colistin	>4	R

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Signature: _____

ANNEXES

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:37:40

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **PaPF09**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:06:13
Receipt Date:20/12/2022 12:06:13
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
NMIC-505	☐	1	Complete	24/12/2022 03:26:52
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:06:14

Organism Name
1 PSEAER Pseudomonas aeruginosa

Comments

Classification
Significant / Unknown

P. aeruginosa.

Drug	PSEAER MIC/*Conc*	SIR
Amikacin	<=4	S
Amoxicillin	>32	R
Amoxicillin-Clavulanate (f)	>32/2	R
Ampicillin	>16	R
Ampicillin-Sulbactam (f)	>4/8	R
Cefazolin	>32	R
Cefepime	2	S
Cefixime	>2	
Cefotaxime	>4	R
Ceftazidime	2	S
Ceftazidime-Avibactam	4/4	S
Ceftolozane-Tazobactam	<=0.5/4	S
Ceftriaxone	>4	R
Cefuroxime	>16	R
Ciprofloxacin	<=0.125	S
Colistin	<=1	X

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

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27/12/2022 10:37:40

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **PaPF09**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:06:13
Receipt Date:20/12/2022 12:06:13
Antimicrobial Therapy:
Auftrag:

Drug	PSEAER MIC/*Conc*	SIR
Ertapenem	>2	R
Fosfomycin w/G6P	32	
Gentamicin	<=1	S
Imipenem	2	S
Levofloxacin	<=0.5	S
Meropenem	0.5	S
Ofloxacin	1	S
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	>4	R
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	2/38	R

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

22/12/2022 09:17:45

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **PaPM01**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:06:24 Receipt Date:20/12/2022 12:06:24
Antimicrobial Therapy:
Auftrag:

Drug	PSEAER MIC/*Conc*	SIR
Ertapenem	>2	R
Fosfomycin w/G6P	32	
Gentamicin	<=1	S
Imipenem	2	S
Levofloxacin	<=0.5	S
Meropenem	0.5	S
Ofloxacin	1	S
Piperacillin-Tazobactam	<=4/4	S
Tigecycline	>4	R
Tobramycin	<=2	S
Trimethoprim-Sulfamethoxazole	2/38	R

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

Page 1/2
27/12/2022 10:28:38

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya
Accession #: **SaPF01**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:07:07
Receipt Date: 20/12/2022 12:07:07
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:07:09

Organism Name		Comments	Classification
1 STAAUE	Staphylococcus aureus		Significant / Unknown

Resistance Markers
1 BLACT Beta-lactamase producing Staphylococcus
1 MRS Methicillin Resistant Staphylococcus

Drug	STAAUE MIC/*Conc*	SIR
Amikacin	<=4	
Ampicillin	>1	R
Cefoxitin	4	R
Ceftaroline	0.25	S
Ciprofloxacin	>4	R
Clindamycin	>1	X
Erythromycin	>2	X
Fosfomycin w/G6P	<=16	
Fusidic Acid	>8	
Gentamicin	<=1	S
Gentamicin-Syn	<=500	
Linezolid	>4	R
Moxifloxacin	>1	R
Mupirocin	>4	

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. Methicilline and Cefoxitine and Oxacilline (MRSA)

CHART REPORT - FINAL

Universitaetsspital Zuerich
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27/12/2022 10:28:38

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **SaPF01**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:07:07 Receipt Date:20/12/2022 12:07:07
Antimicrobial Therapy:
Auftrag:

Drug	STAAUE MIC/*Conc*	SIR
Mupirocin High level	<=256	S
Nitrofurantoin	<=16	S
Oxacillin	>2	R
Penicillin G	>0.25	R
Rifampin	>1	X
Teicoplanin	>4	X
Tetracycline	>2	X
Tobramycin	<=1	
Trimethoprim	>4	X
Trimethoprim-Sulfamethoxazole	>4/76	R
Vancomycin	>4	X

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
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27/12/2022 10:28:38

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **SaPF01**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:07:07 Receipt Date:20/12/2022 12:07:07
Antimicrobial Therapy:
Auftrag:

Drug	STAAUE MIC/*Conc*	SIR
Mupirocin High level	<=256	S
Nitrofurantoin	<=16	S
Oxacillin	>2	R
Penicillin G	>0.25	R
Rifampin	>1	X
Teicoplanin	>4	X
Tetracycline	>2	X
Tobramycin	<=1	
Trimethoprim	>4	X
Trimethoprim-Sulfamethoxazole	>4/76	R
Vancomycin	>4	X

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

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27/12/2022 10:30:05

Patient Name:
Birth Date:
Patient ID:
Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **SaPM02**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:07:37
Receipt Date:20/12/2022 12:07:37
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:07:38

Organism Name		Comments	Classification
1 STAAUE	Staphylococcus aureus		Significant / Unknown

Resistance Markers
1 BLACT Beta-lactamase producing Staphylococcus
1 MRS Methicillin Resistant Staphylococcus

Drug	STAAUE MIC/*Conc*	SIR
Amikacin	<=4	
Ampicillin	>1	R
Cefoxitin	<=2	R
Ceftaroline	<=0.125	S
Ciprofloxacin	<=0.25	S
Clindamycin	>1	X
Erythromycin	>2	X
Fosfomycin w/G6P	<=16	
Fusidic Acid	>8	
Gentamicin	<=1	S
Gentamicin-Syn	<=500	
Linezolid	>4	R
Moxifloxacin	<=0.25	S
Mupirocin	>4	

Signature: _____

X

CHART REPORT - FINAL

Universitaetsspital Zuerich
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Page 2/2
27/12/2022 10:30:05

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **SaPM02**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:07:37 Receipt Date:20/12/2022 12:07:37
Antimicrobial Therapy:
Auftrag:

Drug	STAAUE MIC/*Conc*	SIR
Mupirocin High level	<=256	S
Nitrofurantoin	<=16	S
Oxacillin	>2	R
Penicillin G	>0.25	R
Rifampin	>1	X
Teicoplanin	>4	X
Tetracycline	>2	X
Tobramycin	<=1	
Trimethoprim	>4	X
Trimethoprim-Sulfamethoxazole	>4/76	R
Vancomycin	>4	X

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
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29/03/2023 08:48:15

Patient Name: **Patient ID:'**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Dirk

Accession #: **SaPFO3**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 28/03/2023 15:08:30 Receipt Date:28/03/2023 15:08:30
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
PMIC/ID-88	☑	1	Complete	29/03/2023 01:47:42

Organism Name		Comments	Classification
1 STAAUE	Staphylococcus aureus		Significant / Unknown

Resistance Markers
1 BLACT Beta-lactamase producing Staphylococcus

Drug	STAAUE MIC/*Conc*	SIR
Ampicillin		R
Cefoxitin	<=2	
Ceftaroline	0.25	S
Ciprofloxacin	<=0.5	I
Clindamycin	<=0.25	S
Daptomycin	<=0.5	S
Erythromycin	<=0.25	S
Fosfomycin w/G6P	<=16	S
Fusidic Acid	<=0.5	S
Gentamicin	<=1	S
Gentamicin-Syn	<=500	
Imipenem	<=2	S
Linezolid	1	S
Moxifloxacin	<=0.25	S
Mupirocin High level	<=256	S
Nitrofurantoin	<=16	
Oxacillin	<=0.25	S

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
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29/03/2023 08:48:15

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Dirk

Accession #: **SaPFO3**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 28/03/2023 15:08:30 Receipt Date:28/03/2023 15:08:30
Antimicrobial Therapy:
Auftrag:

Drug	STAAUE MIC/*Conc*	SIR
Penicillin G	>0.25	R
Rifampin	<=0.25	X
Teicoplanin	<=0.5	S
Tetracycline	<=0.5	S
Tigecycline	<=0.25	S
Trimethoprim-Sulfamethoxazole	<=1/19	S
Vancomycin	1	S

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

29/03/2023 08:47:27

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Dirk

Accession #: **SaPFO5**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 28/03/2023 15:09:19 Receipt Date:28/03/2023 15:09:19
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
PMIC/ID-88	☑	1	Complete	29/03/2023 01:49:11

Organism Name		Comments	Classification
1 STAAUE	Staphylococcus aureus		Significant / Unknown

Resistance Markers
1 BLACT Beta-lactamase producing Staphylococcus

Drug	STAAUE MIC/*Conc*	SIR
Ampicillin		R
Cefoxitin	4	
Ceftaroline	0.5	S
Ciprofloxacin	<=0.5	I
Clindamycin	<=0.25	S
Daptomycin	<=0.5	S
Erythromycin	<=0.25	S
Fosfomycin w/G6P	<=16	S
Fusidic Acid	<=0.5	S
Gentamicin	<=1	S
Gentamicin-Syn	<=500	
Imipenem	<=2	S
Linezolid	1	S
Moxifloxacin	<=0.25	S
Mupirocin High level	<=256	S
Nitrofurantoin	<=16	
Oxacillin	0.5	S

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
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29/03/2023 08:47:27

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Dirk

Accession #: **SaPFO5**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 28/03/2023 15:09:19 Receipt Date:28/03/2023 15:09:19
Antimicrobial Therapy:
Auftrag:

Drug	STAAUE	
	MIC/*Conc*	SIR
Penicillin G	>0.25	R
Rifampin	<=0.25	X
Teicoplanin	<=0.5	S
Tetracycline	<=0.5	S
Tigecycline	<=0.25	S
Trimethoprim-Sulfamethoxazole	<=1/19	S
Vancomycin	1	S

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
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29/03/2023 08:46:33

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Dirk

Accession #: **SaPFO7**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 28/03/2023 15:10:15 Receipt Date:28/03/2023 15:10:15
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
PMIC/ID-88	☑	1	Complete	29/03/2023 01:48:57

Organism Name		Comments	Classification
1 STAAUE	Staphylococcus aureus		Significant / Unknown

Resistance Markers
1 BLACT Beta-lactamase producing Staphylococcus

Drug	STAAUE MIC/*Conc*	SIR
Ampicillin		R
Cefoxitin	<=2	
Ceftaroline	0.25	S
Ciprofloxacin	<=0.5	I
Clindamycin	<=0.25	S
Daptomycin	<=0.5	S
Erythromycin	<=0.25	S
Fosfomycin w/G6P	<=16	S
Fusidic Acid	<=0.5	S
Gentamicin	<=1	S
Gentamicin-Syn	<=500	
Imipenem	<=2	S
Linezolid	1	S
Moxifloxacin	<=0.25	S
Mupirocin High level	<=256	S
Nitrofurantoin	<=16	
Oxacillin	<=0.25	S

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

29/03/2023 08:46:32

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Dirk

Accession #: **SaPFO7**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 28/03/2023 15:10:15 Receipt Date:28/03/2023 15:10:15
Antimicrobial Therapy:
Auftrag:

Drug	STAAUE	
	MIC/*Conc*	SIR
Penicillin G	>0.25	R
Rifampin	<=0.25	X
Teicoplanin	<=0.5	S
Tetracycline	<=0.5	S
Tigecycline	<=0.25	S
Trimethoprim-Sulfamethoxazole	<=1/19	S
Vancomycin	1	S

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

29/03/2023 08:46:09

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Dirk

Accession #: **SaPMO8**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 28/03/2023 15:10:39 Receipt Date:28/03/2023 15:10:39
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
PMIC/ID-88	☑	1	Complete	29/03/2023 01:48:49

Organism Name		Comments	Classification
1 STAAUE	Staphylococcus aureus		Significant / Unknown

Resistance Markers
1 BLACT Beta-lactamase producing Staphylococcus

Drug	STAAUE MIC/*Conc*	SIR
Ampicillin		R
Cefoxitin	4	
Ceftaroline	0.5	S
Ciprofloxacin	<=0.5	I
Clindamycin	<=0.25	S
Daptomycin	<=0.5	S
Erythromycin	<=0.25	S
Fosfomycin w/G6P	<=16	S
Fusidic Acid	<=0.5	S
Gentamicin	<=1	S
Gentamicin-Syn	<=500	
Imipenem	<=2	S
Linezolid	1	S
Moxifloxacin	<=0.25	S
Mupirocin High level	<=256	S
Nitrofurantoin	<=16	
Oxacillin	0.5	S

Signature: _____

CHART REPORT - FINAL

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

29/03/2023 08:46:09

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Dirk

Accession #: **SaPMO8**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 28/03/2023 15:10:39 Receipt Date:28/03/2023 15:10:39
Antimicrobial Therapy:
Auftrag:

Drug	STAAUE	
	MIC/*Conc*	SIR
Penicillin G	>0.25	R
Rifampin	<=0.25	X
Teicoplanin	<=0.5	S
Tetracycline	<=0.5	S
Tigecycline	<=0.25	S
Trimethoprim-Sulfamethoxazole	<=1/19	S
Vancomycin	1	S

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

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27/12/2022 10:32:52

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **SaBCSF09**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:07:02 Receipt Date:20/12/2022 12:07:02
Antimicrobial Therapy:
Auftrag:

Test Name	Final	Isolate #	Result	Result Date/Time
MALDI Biotyper ID	☑	1	Complete A: Species Consistency ID for Research Use Only - Not For Diagnostic Use	20/12/2022 12:07:03

Organism Name		Comments	Classification
1 STAAUE	Staphylococcus aureus		Significant / Unknown

Resistance Markers
1 BLACT Beta-lactamase producing Staphylococcus

Drug	STAAUE MIC/*Conc*	SIR
Amikacin	<=4	
Ampicillin		R
Cefoxitin	<=2	
Ceftaroline	0.5	S
Ciprofloxacin	<=0.25	S
Clindamycin	<=0.25	S
Erythromycin	<=0.25	S
Fosfomycin w/G6P	<=16	
Fusidic Acid	<=1	
Gentamicin	<=1	S
Gentamicin-Syn	<=500	
Linezolid	<=0.5	S
Moxifloxacin	<=0.25	S
Mupirocin	<=1	
Mupirocin High level	<=256	S

Signature: _____

CHART REPORT - PRELIMINARY

Universitaetsspital Zuerich
Raemistrasse 100, 8091 Zuerich

27/12/2022 10:32:52

Patient Name: **Patient ID:**
Birth Date: Patient Sex:
Ordering Physician: Unspecified
User Name: Bhavya

Accession #: **SaBCSF09**
Specimen Type: Unspecified
Hospital Service: Unspecified
Collection Date: 20/12/2022 12:07:02 Receipt Date:20/12/2022 12:07:02
Antimicrobial Therapy:
Auftrag:

Drug	STAAUE MIC/*Conc*	SIR
Nitrofurantoin	<=16	S
Oxacillin	<=0.25	S
Penicillin G	>0.25	R
Rifampin	<=0.25	S
Teicoplanin	<=1	S
Tetracycline	>2	X
Tobramycin	<=1	
Trimethoprim	<=1	S
Trimethoprim-Sulfamethoxazole	<=1/19	S
Vancomycin	1	S

Signature: _____