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Ciências da Saúde:

Investigação e Prática



Dr. Guillermo Julián González-Pérez
Dra. María Guadalupe Vega-López
(organizadores)

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PRÓLOGO

Las ciencias de la salud constituyen un campo amplio y dinámico, en permanente transformación, en el que convergen la investigación clínica, la microbiología, la prevención, la gestión de los servicios, las prácticas profesionales y la reflexión crítica sobre la propia construcción del conocimiento en salud. Esta diversidad permite comprender los procesos de salud y enfermedad desde múltiples perspectivas y responder a problemas complejos que involucran tanto dimensiones biológicas como asistenciales, organizacionales, sociales e históricas.

El volumen ***Ciências da Saúde: Investigação e Prática VII*** reúne estudios de autores provenientes de diferentes países - Chile, Colombia, España, Estados Unidos, México, Panamá y Paraguay - que expresan precisamente esta pluralidad de enfoques. Los trabajos aquí presentados abarcan desde la resistencia antimicrobiana y el estudio de biofilms hasta casos clínicos de difícil diagnóstico, enfermedades respiratorias, fragilidad y riesgo social en adultos mayores, enfermería, bienestar laboral, seguridad del paciente e historia de la medicina.

La obra se organiza en tres ejes temáticos.

El primero, Clínica, Microbiología, Enfermedades y Condiciones de Salud, reúne investigaciones centradas en microorganismos de relevancia clínica, resistencia antimicrobiana, enfermedades respiratorias, desafíos diagnósticos y condiciones que inciden en la salud y vulnerabilidad de distintos grupos poblacionales. Los capítulos analizan la actividad antibiofilm frente a *Stenotrophomonas sepilia*, la resistencia de cepas bacterianas aisladas de ambientes nosocomiales, un posible caso de enfermedad de Creutzfeldt-Jakob con presentación atípica, factores asociados a la bronquitis infantil y la fragilidad y el riesgo social en adultos mayores de dos instituciones de atención. En conjunto, estos trabajos ponen de manifiesto la importancia de la vigilancia microbiológica, el diagnóstico diferencial, la evaluación clínica y el análisis integral de factores biológicos y sociales relacionados con la salud.

El segundo eje, Prácticas Profesionales, Gestión y Calidad en Salud, aborda la organización del trabajo sanitario y las condiciones necesarias para fortalecer la calidad de la atención y el bienestar de quienes integran los servicios y las instituciones. Los estudios analizan el desarrollo de la enfermería de práctica avanzada en el ámbito laboral, la relación entre prácticas organizacionales saludables, compromiso y bienestar laboral, así como el impacto de herramientas de gestión de la calidad sobre la seguridad del paciente. Estos trabajos muestran que la calidad en salud depende no

solo de procedimientos clínicos, sino también de competencias profesionales, liderazgo, organización institucional, prevención de riesgos y mejora continua.

El tercer eje, Historia, Epistemología e Interpretación de la Enfermedad, incorpora una dimensión reflexiva al volumen mediante el estudio de episodios históricos que continúan planteando interrogantes para la medicina contemporánea. A través del análisis de la coreomanía de Estrasburgo de 1518 y del denominado sudor inglés, los capítulos examinan los límites del diagnóstico retrospectivo, la relación entre enfermedad, cultura y contexto histórico, y las dificultades epistemológicas de atribuir causas precisas a fenómenos médicos del pasado cuando la evidencia disponible es incompleta.

La integración de estos tres ejes ofrece una visión amplia de la investigación y de la práctica en salud. La microbiología y la clínica dialogan con el estudio de condiciones sociales de salud, la gestión, la enfermería y la seguridad asistencial, mientras que la historia y la epistemología recuerdan que el conocimiento médico se construye a partir de evidencias, interpretaciones y contextos que también deben ser examinados críticamente.

Esperamos que este volumen contribuya a la difusión de investigaciones relevantes, al intercambio entre profesionales y académicos de diferentes latitudes y al fortalecimiento de prácticas orientadas a una atención en salud cada vez más segura, rigurosa y fundamentada.

Dr. Guillermo Julián González-Pérez

Dra. María Guadalupe Vega-López

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ANTIBIOFILM ACTIVITY OF VG111 AGAINST *STENOTROPHOMONAS SEPIILIA*: A NOVEL APPROACH TO BIOFILM ERADICATION

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ABSTRACT: *Stenotrophomonas sepilia* is a recently described member of the *Stenotrophomonas maltophilia* complex (Smc) that is increasingly recognized in human infections. Since its first description in 2021, *S. sepilia* has been recovered from diverse clinical specimens and has emerged as an important clinical species, with increasing evidence of strong biofilm-forming capacity. Biofilm formation may contribute to persistence and reduced antimicrobial efficacy, highlighting the need for alternative or complementary strategies targeting established biofilms. VG111 is a polyherbal formulation developed originally as a wound-healing agent and subsequently shown to possess antibacterial and antibiofilm activities against several clinically important pathogens. This chapter reviews the available evidence regarding the activity of VG111 against *S. sepilia*, with particular emphasis on its effects on preformed biofilms. Clinical isolates of *S. sepilia* have demonstrated robust biofilm production, with the isolates evaluated by the Gautam and collaborating laboratories exhibiting a strong-biofilm phenotype. VG111 demonstrated concentration-dependent antibacterial activity against *S. sepilia*: 5% (v/v) substantially inhibited bacterial growth, whereas 10% (v/v) produced complete inhibition; an MIC of 2 mg/mL was also reported to completely inhibit growth. Importantly, VG-111 exhibited activity against

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established *S. sepilia* biofilms. Treatment with 25% (v/v) VG-111 significantly disrupted preformed biofilms, while concentrations ranging from 25% to 100% (v/v) were associated with reductions in biofilm-associated viable cells and total biomass. Collectively, these findings indicate that VG-111 possesses both antibacterial and biofilm-disrupting activity against *S. sepilia* and support its further investigation as a potential topical adjunctive strategy for biofilm-associated infections. Future studies should define the molecular basis of biofilm disruption, assess activity across genetically diverse clinical isolates, distinguish biofilm prevention from eradication, and establish formulation consistency, safety, and efficacy in appropriate in vivo and controlled clinical studies.

KEYWORDS: Stenotrophomonas; diabetic foot ulcers; pressure ulcers; wound infections; biofilm.

1. INTRODUCTION

Stenotrophomonas is a genus of aerobic, non-fermenting, Gram-negative bacilli. Taxonomically, the genus Stenotrophomonas is classified within the family Lysobacteraceae (the Xanthomonas group) [Yu et al., 2024; Zhao et al., 2024]. Box 1 provides the detailed taxonomic classification of the genus Stenotrophomonas. As of December 2025, the genus Stenotrophomonas includes 31 classified species, along with more than 3044 other unclassified species, ubiquitously distributed across a range of environmental habitats, with soil and plant-associated niches serving as their primary natural reservoirs [Yu et al.,2024].

Box 1: Taxonomic Classification of Stenotrophomonas.

Taxonomic classification#:
Domain: Bacteria
Kingdom: Pseudomonadati
Phylum: Pseudomonadota
Class: Gammaproteobacteria
Order: Lysobacterales (earlier known as Xanthomonadales)
Family: Lysobacteriaceae (previously Xanthomonadaceae)
Genus: Stenotrophomonas
Species: Stenotrophomonas maltophilia (Type species)
Stenotrophomonas sepilia and 29 other classified species, and more than 3044 unclassified species (as described in the National Center for Biotechnology Information (NCBI) taxonomy (https://www.ncbi.nlm.nih.gov/datasets/taxonomy/))

*Yu et al.,2024; An et al., 2018; Coutinho et al., 2022; Li et al.,2024; Kumar et al., 2029.

Although the genus Stenotrophomonas includes many classified and unclassified environmental species, there are medically important Stenotrophomonas species as

well, with *S. maltophilia* being the most prevalent. Based on phylogenetic closeness as indicated by 16S rDNA sequence similarity, the closest species are grouped within the *Stenotrophomonas maltophilia* complex (Smc). *S. maltophilia*, the type species of the Smc, remains the most prevalent, followed by *S. seipilia*, a newly described species, and others. Smc isolates exhibit high intra-species heterogeneity and greater genomic plasticity due to their increased natural ability to take up DNA, a high rate of genomic reassortment, and remarkable hypermutating activity [Pinski *et al.*, 2020; Berg *et al.*, 2015]. Such high intra-specific heterogeneity and genomic plasticity contribute to the emergence of multidrug resistance (MDR) in the isolates (both intrinsically and extrinsically) and to the genetic basis of their virulence, including biofilm-forming ability.

Moreover, the recent rise in the isolation of Smc isolates from both community- and hospital-associated infections worldwide has made these pathogens an emerging concern [Li *et al.*, 2024; Jiang *et al.*, 2022; Said *et al.*, 2023; Chakraborty *et al.*, 2024]. Infections caused by these MDR Smc isolates are difficult to treat with conventional antimicrobial regimens and are associated with longer hospitalizations, higher healthcare costs, increased disease severity, and mortality [Prawang *et al.*, 2022].

Such widespread antimicrobial resistance warrants new therapeutic options. Considerable efforts from all parts of the world are being directed toward identifying alternative strategies to limit or prevent the emergence of resistance. In this context, a wide range of naturally derived antimicrobial compounds has been investigated for their potential therapeutic applications, including (poly)herbal formulations with antimicrobial activity. The greatest benefit of using (poly)herbal agents or their phytochemicals as antimicrobial agents is molecular promiscuity, the ability to interact with or act on multiple types of molecules in bacterial cells [Guedes *et al.*, 2024]. Conventional antibiotics often exert their effects by targeting a specific bacterial target, whereas phytochemicals in polyherbal compounds may act through multiple molecular pathways and interact with several bacterial proteins simultaneously [Guedes *et al.*, 2024; Gray *et al.*, 2020; Wink *et al.*, 2022]. Such broad target engagement can make it more difficult for bacteria to adapt through a single resistance mechanism, potentially reducing the likelihood of resistance emerging against these compounds [Guedes *et al.*, 2024].

VG111 is a polyherbal formulation originally developed as a natural wound-healing remedy. The Gautam Lab at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, developed and formulated it (patent no. 353978, accessible through <https://iprsearch.ipindia.gov.in/publicsearch>) [Singh *et al.*, 2024]. The formulation is based on traditional Indian medicine, Ayurveda, dating back to ancient

times, and incorporates multiple herbs commonly found in the lower Himalayan and Himalayan regions [https://iprsearch.ipindia.gov.in/publicsearch, Singh *et al.*, 2024]. Subsequent work by the Gautam Lab on VG111 has demonstrated antimicrobial and anti-biofilm activity, along with remarkable wound-healing capability. The efficacy of VG111 as a wound-healing, antibacterial, and antibiofilm agent has been explored and established in recent works from the Gautam Lab, and these works are available in the public domain [Singh *et al.*, 2024; Singhal *et al.*, 2024]. In the current chapter, the efficacy of VG111 as an antibiofilm agent and its impact on *Stenotrophomonas sepilia* biofilms have been thoroughly discussed.

2. STENOTROPHOMONAS SEPILIA: DISCOVERY OF A NEW SPECIES

Stenotrophomonas sepilia (*S. sepilia*) is a newly described species within the genus *Stenotrophomonas*. It was first reported in 2021. The organism was isolated from the blood culture of a 28-year-old male patient undergoing cardiothoracic vascular surgery at a tertiary care hospital in Chandigarh, a northern Indian city (30.7650° N, 76.7750° E) [Gautam *et al.*, 2021].

The isolate was obtained from the 16975th blood culture among 46243 blood cultures performed between April 2012 and March 2013 and was one of 33 *S. maltophilia* isolates [Gautam *et al.*, 2021]. Initially identified as *S. maltophilia*, this isolate exhibited a fatty acid profile distinct from that of the type strain *S. maltophilia* ATCC 13637^T (Table 1). Subsequent extensive biochemical characterization also revealed marked differences from the same type strain (*S. maltophilia* ATCC 13637^T) (Table 2). Based on these distinctive biochemical features, the strain was designated SM16975 [Gautam *et al.*, 2021].

Table 1: Cellular fatty acid profile of *Stenotrophomonas sepilia* SM16975^T compared with the type strain *Stenotrophomonas maltophilia* ATCC13637^T.

Fatty acid [#]	<i>S. sepilia</i> SM16975 ^T	<i>S. maltophilia</i> ATCC 13637 ^T
Straight-chain fatty acids		
C10:0	0.68	1.49
C14:0	2.43	3.58
C16:0	10.83	12.14
C18:0	0.32	2.12
Branched-chain fatty acids		
iso-C11:0	3.45	3.04
iso-C13:0	0.36	0.21
iso-C14:0	0.70	0.35
anteiso-C11:0	0.12	0.13
anteiso-C13:0	0.22	–

Fatty acid [#]	<i>S. seipilia</i> SM16975 ^T	<i>S. maltophilia</i> ATCC 13637 ^T
iso-C15:0	36.92	23.47
iso-C15:1 F	0.63	0.82
anteiso-C15:0	15.42	10.16
iso-C16:0	1.69	0.68
iso-C17:0	4.27	2.91
anteiso-C17:0	0.54	0.39
iso-C19:0	0.27	0.32
Unsaturated fatty acids		
C14:1 ω5c	0.10	0.22
C16:1 ω9c	1.49	5.66
C17:1 ω8c	0.13	–
C18:1 ω9c	1.06	3.21
C18:3 ω6c	–	–
Hydroxy fatty acids		
C10:0 3-OH	0.13	0.19
C12:0 3-OH	2.32	3.05
C13:0 2-OH	0.51	0.24
iso-C11:0 3-OH	1.75	1.14
iso-C13:0 3-OH	2.75	1.69

Table adapted from Gautam V *et al.* 2021 [Gautam *et al.*, 2021].

[#]Values are percentages of total cellular fatty acids. “–” indicates that the component was not detected or reported.

Table 2: Comparison of biochemical characteristics[#] of *Stenotrophomonas seipilia* SM16975^T and the type strain *Stenotrophomonas maltophilia* ATCC13637.

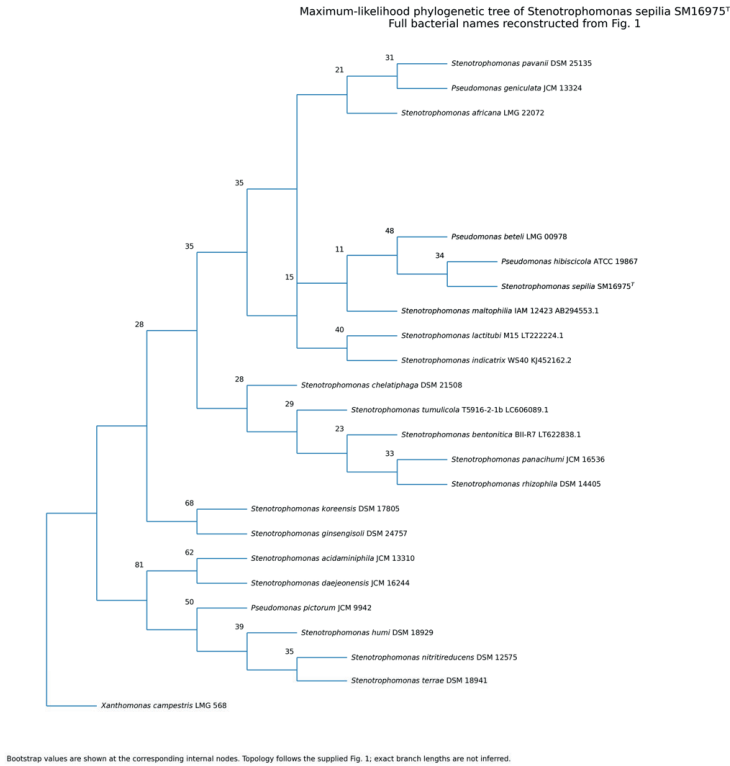
Biochemical	<i>S. seipilia</i> SM16975 ^T	<i>S. maltophilia</i> ATCC 13637 ^T
Sucrose	+	–
pH 5	+	–
N-Acetyl-β-D-Mannosamine	–	+
D-Galactose	–	+
L-Rhamnose	–	+
Fusidic acid	–	+
D-Glucose-PO4	–	+
L-Histidine	–	+
Pectin	–	+
Nalidixic Acid	–	+
β-Hydroxy-D, L-Butyric Acid	–	+
Formic Acid	–	+

Table showing only the distinctive characteristics, adapted from Gautam V *et al.*, 2021 [Gautam *et al.*, 2021].

[#]Biochemical characterization was performed using the BIOLOG GEN III MicroPlate on the OmniLog GEN III system.

Whole-genome analyses of strain SM16975 (NCBI Accession number LXXZ00000000) revealed a genome length of 4,582,512 bp, compared with the 4,989,212 bp annotated circular genome of *S. maltophilia* ATCC 13637. The measured G+C content was 66.4% for strain SM16975, compared with 66.1% for *S. maltophilia* ATCC 13637. Further genome-based comparison of SM16975 and the type strain *S. maltophilia* ATCC 13637 showed a genomic similarity of slightly more than 91%, as revealed by average nucleotide identity (ANI) and OrthoANI analyses [Gautam *et al.*, 2021; Davenport *et al.*, 2014]. Marked distinctness scores were also obtained for average amino acid identity (AAI; 94.07%) and digital DNA-DNA hybridization (dDDH; 44.3%). The core-genome-based phylogenetic analysis placed strain SM16975 in a separate clade from the type strain *S. maltophilia* ATCC 13637 (Figure 1) [Gautam *et al.*, 2021].

Figure 1: Phylogenetic tree depicting the maximum likelihood based on the alignment of the 16S rRNA gene sequence of *Stenotrophomonas sepilia* SM16975T with various type strains of the genus *Stenotrophomonas* and other closely related misclassified member species of the genus *Pseudomonas* [Gautam *et al.*, 2021].



Based on the fatty acid profile, biochemical characteristics, and genomic analyses, strain SM16975 was assigned species status, i.e., *Stenotrophomonas sepilia*, within the

genus *Stenotrophomonas*. The species name “*sepihia*” is attributed to sepsis, linking it to its discovery following isolation from blood.

However, the 16S rRNA of SM16975 showed 99.58% identity with *S. maltophilia* ATCC 13637, placing *S. sepihia* within the Smc along with *S. maltophilia*, *Pseudomonas hibiscicola*, and about 25 other unclassified species, including the recently proposed *S. veracruzansensis* and *S. mexicanensis* [Yu *et al.*, 2024, Gautam *et al.*, 2021].

3. *STENOTROPHOMONAS SEPIHIA*, A NEW SPECIES IN THE GENUS *STENOTROPHOMONAS* WITH MEDICAL IMPORTANCE

As discussed earlier in the chapter, the genus *Stenotrophomonas* includes many environmental species and a few medically relevant species associated with human infections. Medically important members include *S. maltophilia*, *S. africana*, and, since 2021, *S. sepihia*.

Most recently, in 2025, *S. geniculate* and *S. indicatrix* were reported as two additional human pathogens in the genus *Stenotrophomonas* [Ortiz *et al.*, 2025]. However, *S. maltophilia* remains the primary pathogenic species and is etiologically associated with a wide range of human infections. *S. africana* has also been sporadically associated with primary meningoencephalitis in an HIV-seropositive individual [Drancourt *et al.*, 1997]. Following the first report in 2021, *S. sepihia* has been linked to a wide range of infections in both immunocompromised and immunocompetent individuals. It has been isolated from a wide range of clinical specimens, including blood (predominantly), respiratory specimens (sputum, endotracheal aspirate, and pleural fluid), pus, and biliary drainage. It has been found to cause infections as a sole pathogen (monomicrobial infections) or as a co-infecting agent (in polymicrobial infections).

A 2024 study from the Gautam Lab has already shown that despite its 2021 discovery, *S. sepihia* has become the second most prevalent species in India, where it was discovered, in association with human infections [Chakraborty *et al.*, 2024]. Another recent study by Li *et al.* (2024), which included isolates from six continents (Asia, Europe, Africa, North America, South America, and Oceania), also found that *S. sepihia* is already the second most prevalent species in the genus *Stenotrophomonas* worldwide. The study highlighted the markedly higher proportion of *S. sepihia* in Asia and Europe, after *S. maltophilia*, among the included *Stenotrophomonas* isolates. Africa had the highest proportion of *S. sepihia*, surpassing *S. maltophilia* (often considered the most prevalent species) [Li *et al.*, 2024].

4. PATHOGENICITY AND VIRULENCE OF THE NEW SPECIES

S. sepilia has already been observed to have pathogenic potential and an etiological association with a wide range of infections in both immunocompetent and immunocompromised individuals. Clinical isolates of *S. sepilia* have been recovered from a spectrum of clinical specimens in both monomicrobial and polymicrobial infections. These findings already support the pathogenic capabilities of the new species. However, the Gautam Lab has experimentally assessed the pathogenicity of *S. sepilia* using a *Caenorhabditis elegans* model. In slow-killing assays, the pathogenicity of *S. sepilia* was comparable to that of *S. maltophilia* [unpublished data; Gautam Lab]. However, the complete range of virulence factors remains to be determined, and *S. sepilia* has been found to produce biofilm.

5. BIOFILM PRODUCTION ABILITY OF *S. SEPILIA*

Numerous studies have shown that most *S. maltophilia* isolates are biofilm producers. However, non-biofilm-producing isolates also exist. Among the biofilm producers, isolates are classified as strong, moderate, or weak producers, depending on the degree of biofilm production [Bostanghadiri *et al.*, 2019; Azimi *et al.*, 2020].

Because *S. sepilia* is a newly established species, there are not many public reports that demonstrate and evaluate its biofilm-forming ability. However, since the first report in 2021, a few laboratories, including the Gautam Lab and Patil Lab in India, have been studying the biofilm-forming ability and virulence of clinical isolates of *S. sepilia* from several geographical areas across India. Studies from both labs, conducted in collaboration, have found that 100% of clinical isolates of *S. sepilia* are strong biofilm producers (unpublished data; Figures 2 & 3). Recently, Li K. *et al.* (2024) studied the motility and biofilm production of isolates of *S. sepilia* from six continents (Asia, Europe, North America, South America, Africa and Oceania) and reported that *S. sepilia* exhibits superior performance in both traits compared with *S. maltophilia* (the type species in Smc) and other closely related species of the genus *Stenotrophomonas* [Li *et al.*, 2024].

Figure 2: Biofilm produced by clinical isolates of *Stenotrophomonas sepipla* (stained with 0.1% crystal violet). (a) Biofilm formed by *S. sepipla* adhering to the wall of the well (side view). (b) *S. sepipla* biofilm at the bottom of the wells.

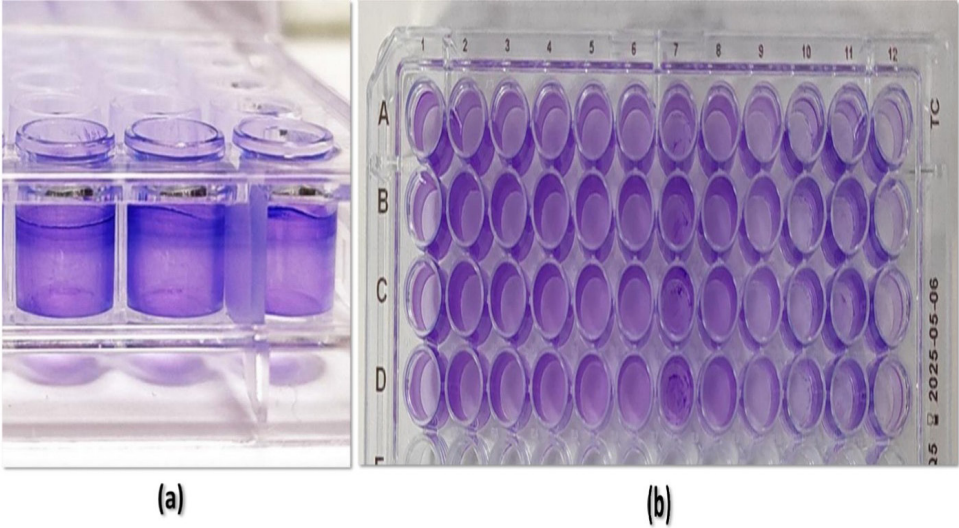
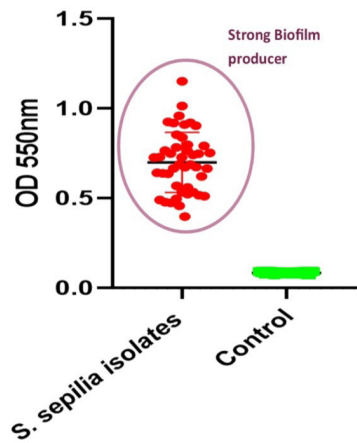


Figure 3: Degree of biofilm production among 46 clinical isolates of *S. sepipla*. All were strong biofilm producers.



The strong biofilm-forming ability of *S. sepipla* is further enhanced by iron supplementation. Iron is an important signaling molecule that can simultaneously enhance the expression of various matrix-remodeling genes and decrease the expression of genes associated with mobility (unpublished data; Gautam Lab).

Numerous studies have shown that bacterial biofilms are associated with persistent infections, longer hospital stays, and the development of antimicrobial resistance [Costerton *et al.*, 1999; Singh *et al.*, 2021; Andrés-Lasheras *et al.*, 2024].

Isolates of Smc produce biofilms to varying degrees. Studies from different parts of the world have reported a significant association between antimicrobial resistance (AMR) and biofilm-producing capacity in *S. maltophilia* [Azimi *et al.*, 2020]. Such an association is also expected in clinical isolates of *S. sepilia*, given its genetic closeness to *S. maltophilia* and its similar environmental niche. Given the ability of clinical isolates of *S. sepilia* to produce robust biofilms, exploring newer and more effective anti-biofilm agents is warranted. In this context, a natural product with simultaneous antimicrobial and anti-biofilm activity may hold promise.

6. VG-111, THE ANTIBIOFILM AGENT

VG-11 has been shown to effectively heal wounds in experimental animal models. Subsequently, its efficacy was established across a spectrum of soft- and deep-tissue wounds with ulceration in human patients and in companion animals. Most notably, it has demonstrated the ability to resolve chronic wounds, especially diabetic foot ulcers (DFUs) and chronic bedsores or pressure ulcers (PPUs) [Singh *et al.*, 2026]. Beyond its wound-healing properties, VG-111 showed pronounced antibacterial activity at concentrations of 2.5-5.0% (v/v), with inhibitory effects against clinically significant drug-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), colistin-resistant *Escherichia coli*, *Acinetobacter baumannii*, and several other priority bacterial pathogens [Singh *et al.*, 2024; Singhal *et al.*, 2024]. Efforts have been made to identify the principal bioactive compound(s) in the formulation that may confer antimicrobial activity; however, a single bioactive compound responsible for its antimicrobial action could not be identified. The chromatogram obtained from high-performance liquid chromatography (HPLC) indicates that the action may be due to the synergism of multiple active compounds [Singhal *et al.*, 2024].

However, the recent study by Singh *et al.* (2026), conducted in collaboration with the Gautam Lab, has identified 41 chemical constituents in VG111 using gas chromatography-mass spectrometry (GC-MS). These findings were further complemented by *in silico* molecular docking simulations that indicated mechanisms underlying VG111's profound antimicrobial and antibiofilm potential [Singh *et al.*, 2026].

Figure 4: Wound over the plantar aspect, dorsum of right mid foot, over medial malleolus and lateral aspect of right heel (Biofilm-associated infection in DFU).



However, in addition to its comprehensive wound-healing and antimicrobial capabilities, VG-111 demonstrated a marked ability to disrupt biofilms produced by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and other organisms. This antibiofilm activity has also been demonstrated in biofilm-associated open wounds in experimental animal models and in companion dogs. In clinical practice, as an oil-based topical agent, it has shown visible anti-biofilm activity in improving and resolving chronic wounds ex vivo that invariably develop biofilms [Singh *et al.*, 2024; Singhal *et al.*, 2024].

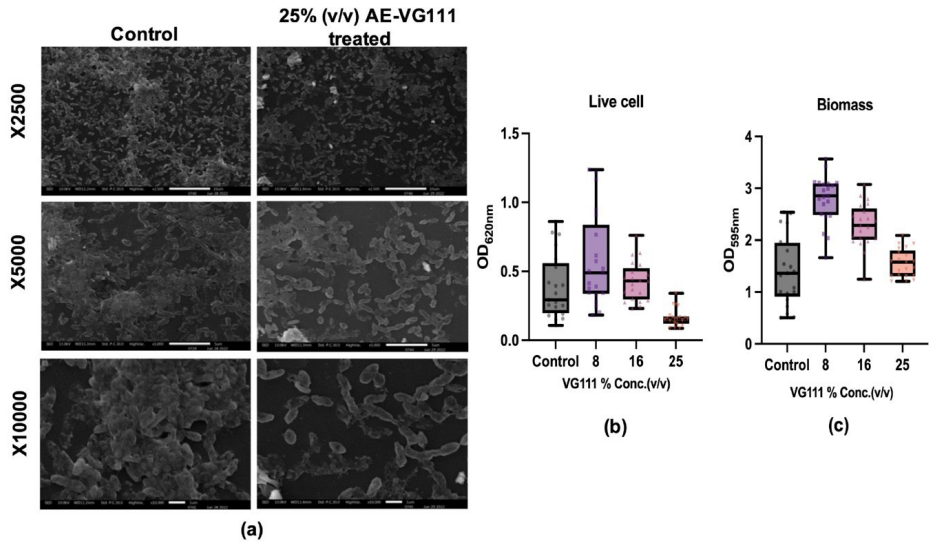
7. IMPACT OF VG-111 AGAINST *S. SEPIILIA* BIOFILM

The efficacy of VG-111 was also confirmed against the new pathogen *S. sepilia*. 5% (v/v) VG-111 in culture media substantially inhibited *S. sepilia* growth, and 10% (v/v) produced complete inhibition. Furthermore, a minimum inhibitory concentration (MIC) of 2 mg/mL resulted in complete inhibition of *S. sepilia* [unpublished data; Gautam Lab]. This concentration is acceptable for a topical agent derived from complex natural extracts.

Earlier, the clinical isolates of the new species *S. sepilia* were noted to have robust biofilm-forming ability. Biofilms protect bacteria from environmental insults, reduce treatment efficacy, and confer resistance to antimicrobial agents. Although all these strengthening aspects of biofilms in *S. sepilia* remain to be explored through rigorous experimental methods, the ability of isolates of a new pathogen species to produce strong biofilms necessitates the exploration of newer anti-biofilm agents.

VG-111 was capable of disrupting the strong biofilms that *S. sepilia* produced in vitro. A 25% (v/v) concentration of VG-111 significantly disrupted pre-formed biofilms produced by *S. sepilia*. The impact on pre-formed biofilms (by *S. sepilia*) was notable, with a reduction in live cells within the biofilm matrix and overall biomass after treatment with VG-111 at concentrations between 25% and 100% (v/v). Scanning electron microscopy (SEM) clearly showed the diminished and disrupted pre-formed biofilm by *S. sepilia* (Figure 5).

Figure 5: *S. sepilia* biofilm treated with 25% AE (aqueous extract) of VG111 at 2500x, 5000x, and 10000x magnification; concentration-dependent mature biofilm clearance shown using (b) live cells (tetrazolium-chloride staining) and (c) biomass (crystal-violet staining).



8. SUMMARY

S. sepilia has emerged as an important member of the *S. maltophilia* complex (Smc) since its initial description in 2021. The strong biofilm-forming phenotype described for clinical *S. sepilia* isolates therefore represents an important biological characteristic rather than merely an in vitro phenotype. The present chapter summarizes observations indicating that clinical isolates of *S. sepilia* are strong biofilm producers. This finding is consistent with the broader biology of the Smc, in which biofilm formation is an important component of persistence and survival. Studies of *S. maltophilia* have demonstrated that biofilm-associated cells can exhibit substantially reduced susceptibility to antimicrobial agents compared with planktonic cells. Importantly, biofilm-associated antimicrobial tolerance is multifactorial and may involve restricted antimicrobial penetration, altered metabolic states, physiological heterogeneity, and protection provided by the extracellular

matrix. Consequently, conventional susceptibility testing of planktonic bacteria may not fully predict the response of an established biofilm to antimicrobial intervention.

This consideration provides a strong rationale for investigating agents that can directly affect the biofilm state. In this context, VG-111 is particularly interesting because its reported activity is not restricted to conventional antibacterial effects. VG-111 was originally developed as a natural wound-healing formulation, and subsequent work has reported antimicrobial, antibiofilm, and wound-healing activity in experimental and clinical settings. A 2024 clinical case series involving different wound types reported wound infection clearance, reduction or disappearance of exudate, granulation and epithelialization, together with antibiofilm activity during treatment with VG-111. A subsequent publication from the Gautam Lab also described VG-111 as a formulation with anti-pathogenic activity and accelerated wound healing in humans and companion animals [Singhal *et al.*, 2024; Singh *et al.*, 2026].

The findings presented here extend these observations to the newly recognized pathogen *S. sepilia*. VG-111 demonstrated measurable antibacterial activity against *S. sepilia*, with 5% (v/v) substantially inhibiting bacterial growth and 10% (v/v) producing complete inhibition; an MIC of 2 mg/mL was also reported to produce complete inhibition. More importantly, VG-111 was able to act against preformed *S. sepilia* biofilms. Treatment with 25% (v/v) VG-111 significantly disrupted established biofilms, while concentrations ranging from 25% to 100% (v/v) were associated with reductions in viable cells within the biofilm and overall biofilm biomass.

The ability to affect an already established biofilm is particularly noteworthy. Biofilm eradication is inherently more difficult than inhibition of initial attachment or prevention of biofilm development because mature biofilms contain spatially organized bacterial populations embedded within an extracellular matrix and frequently include physiologically heterogeneous cells with different metabolic states. Such characteristics contribute to antimicrobial tolerance and persistence. Thus, the observed reduction in both viable cells and biofilm biomass following VG-111 exposure suggests that its activity may extend beyond inhibition of planktonic growth to interference with the established biofilm community.

The distinction between antibacterial activity and antibiofilm activity is also important. A reduction in biofilm biomass may partly result from killing or inhibition of bacterial cells rather than from a direct effect on biofilm matrix components. Conversely, the SEM observations of altered preformed biofilm architecture suggest a structural effect, but microscopy alone cannot establish whether VG-111 directly targets the extracellular

matrix, bacterial adhesion, cell-cell signaling, or cellular viability. Future studies using viable-cell enumeration, metabolic activity assays, confocal laser scanning microscopy, extracellular polymeric substance quantification, transcriptomics/proteomics, and time-kill analyses would help distinguish these mechanisms.

The potential clinical relevance of VG-111 is nevertheless considerable. Biofilm-associated infections are difficult to manage because biofilm cells can withstand antimicrobial exposure and persist despite apparently appropriate therapy. This is especially relevant to *S. sepilia*, for which emerging evidence indicates strong biofilm-forming capacity and increasing clinical recognition. An agent capable of combining antibacterial and biofilm-disrupting properties could therefore offer a complementary approach to conventional antimicrobial treatment, particularly in localized or wound-associated infections where topical delivery may be feasible.

9. CONCLUSION

Overall, VG-111 is a promising candidate for the topical antibiofilm strategy against *S. sepilia*, particularly where biofilm-associated persistence complicates infection management. Future studies should focus on defining its molecular and cellular mechanisms of biofilm disruption, determining the contribution and possible synergy of individual phytochemical constituents, evaluating activity across genetically diverse clinical isolates, assessing biofilm prevention versus established-biofilm eradication, and establishing efficacy, safety, formulation consistency, and pharmacological feasibility in appropriate in vivo and controlled clinical studies.

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