REVIEW ARTICLE

Deciphering the Clinical Significance of *Stenotrophomonas sepilia* in the *Stenotrophomonas maltophilia* Complex

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Abstract

Stenotrophomonas sepilia is a newcomer in the bacterial world, emerging as an important player in hospital-acquired infections. First discovered in 2021 from a patient in Chandigarh, India, this bacterium is part of a larger family [the *Stenotrophomonas maltophilia* complex (Smc)] known for its natural resistance to many antibiotics. Like its relatives, *S. sepilia* has several tricks up its sleeve, including the ability to form biofilms, pump out antibiotics, and break down drugs, making it a tough adversary in clinical settings.

What makes *S. sepilia* particularly worrisome is its growing resistance profile. Standard first-line antibiotics such as ceftazidime and trimethoprim-sulfamethoxazole sometimes fall short, while newer drugs like levonadifloxacin show promise in lab tests. Notably, minocycline has emerged as a reliable treatment option, proving effective against all tested isolates. This highlights an urgent need to fine-tune treatment strategies specifically for this emerging pathogen.

Epidemiologically, the bacterium is making its mark, especially in India, where it accounts for 31–50% of isolates in major hospitals. Beyond India, its presence is significant in Africa and is steadily increasing in Asia and Europe, though it remains less common in North and South America. This global spread calls for tailored infection control measures and robust surveillance to keep track of its evolution and resistance patterns.

In the lab, *S. sepilia* has shown formidable virulence. It not only forms strong biofilms and moves rapidly but also manages to persist on hospital surfaces and medical devices. Studies using the nematode *Caenorhabditis elegans* have shown that this bacterium can be just as deadly as *S. maltophilia*, with infected nematodes succumbing within 216 hours. These findings underscore its potential to cause serious infections, especially in vulnerable patients.

To better detect and distinguish *S. sepilia* from its relatives, researchers have developed a specific polymerase chain reaction (PCR) test targeting a unique region of its genome. This advancement in diagnostic technology means that clinicians can now identify the bacterium more accurately and act quickly to manage infections.

In summary, *S. sepilia* is a rapidly emerging pathogen with a complex resistance profile and significant virulence. Its growing role in hospital infections, combined with its multidrug-resistance (MDR), makes it a formidable challenge for healthcare providers. Moving forward, it is crucial to invest in research that uncovers its resistance mechanisms, refines treatment options, and improves diagnostic methods. Alongside improved infection control and antimicrobial stewardship, innovative strategies such as bacteriophage therapy and quorum-sensing inhibitors might offer new hope in the fight against this resilient pathogen.

Keywords: Biofilm formation, Multidrug-resistance, Nosocomial infections, *Stenotrophomonas sepilia*.

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Introduction

Nonfermenting Gram-negative bacteria (NFGNB) are now being recognized as serious threats in hospitals. These pathogens are capable of causing severe and potentially fatal infections. These bacteria, characterized by their inability to ferment carbohydrates, are highly adaptable and formidable in clinical settings. Among the prominent NFGNB, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia* have garnered considerable attention for their role in nosocomial infections. Their persistence and prevalence in healthcare settings underscore the urgent need for robust infection control measures and advanced clinical research.[1]

The pathogenicity of *S. maltophilia* is closely tied to its ability to form biofilms on surfaces such as medical devices and host tissues. These biofilms act as physical and chemical barriers, shielding the bacteria from antimicrobials and host immune defenses. Additionally, intrinsic resistance mechanisms—including low outer membrane permeability,[2–4] efflux pumps,[5–11] bacterial metabolic machinery,[12,13] and antibiotic-modifying enzymes[14–19]—further complicate treatment efforts, rendering the bacterium resistant to many conventional antibiotics.[20–22] These traits have established *S. maltophilia* as a formidable opportunistic pathogen in modern healthcare systems.[23–25]

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Recent advances in genomics have highlighted that S. *maltophilia* is part of a cryptic group of closely related species collectively termed the *S. maltophilia* complex (Smc). This group includes species such as *Stenotrophomonas africana*,[26] *Stenotrophomonas pavanii*, and *Stenotrophomonas sepilia*, etc. each with distinct ecological niches and clinical relevance. While traditional phenotypic and biochemical methods often fall short in distinguishing these species, genomic tools, including whole-genome sequencing and average nucleotide identity (ANI) analysis, have refined their taxonomy and provided a clearer picture of their global distribution and pathogenic potential.

Interestingly, however, research has also shown that certain strains of *S. sepilia* (e.g., *S. sepilia* ZH16) can display robust plant-growth-promoting capacities—such as solubilizing critical nutrients like phosphate and zinc and producing the plant hormone indole-3-acetic acid (IAA)—when isolated from agricultural settings.[27] These findings suggest that *S. sepilia* is not solely an emerging clinical threat but can also confer agronomic benefits, particularly in rice farming, through enhanced root colonization and stress tolerance mechanisms. While this dual identity reinforces the adaptability of *S. sepilia*, it also raises questions regarding how environmental factors and genetic plasticity might shape its pathogenic potential vs its utility in sustainable agriculture.

Among the members of the Smc, *S. sepilia* has gained particular prominence. First isolated from a patient in Chandigarh, India, in 2021, this novel species has demonstrated significant clinical and geographical prevalence. It was identified during genomic investigations aimed at refining the taxonomy of the Smc, emphasizing its genetic distinction and clinical relevance.[28] The rising incidence of *S. sepilia* in nosocomial infections, coupled with its multidrug-resistant (MDR) profile, underscores its expanding role as a public health threat. Like its other species within the Smc, *S. sepilia* exhibits resistance through biofilm formation, efflux systems, and antibiotic-degrading enzymes, with enhanced resistance to first-line therapies.

The growing prevalence of MDR infections caused by S. *sepilia* has necessitated the exploration of novel therapeutic options. Levonadifloxacin, a next-generation fluoroquinolone, has shown promise in combating Gram-positive and Gram-negative pathogens, including resistant strains of *S. maltophilia*. Preliminary studies indicate its efficacy against *S. sepilia*, paving the way for further comparisons with established treatments like levofloxacin to identify optimal therapeutic.[29]

Understanding the epidemiology, virulence factors, and genomic adaptability of *S. sepilia* is critical in addressing the challenges posed by this emerging pathogen. Comprehensive genome studies of Smc members provide valuable insights into resistance mechanisms, biofilm formation, and survival strategies, all of which are instrumental in advancing diagnostic and therapeutic approaches. This review aims to elucidate the taxonomy, clinical significance, resistance patterns, and therapeutic challenges associated with *S. sepilia*, emphasizing the urgent need for innovative solutions to combat this pathogen and mitigate its impact on public health.

Addressing the challenges posed by *S. sepilia* requires a multifaceted strategy. The development of novel diagnostic tools, such as polymerase chain reaction (PCR)-based assays and whole-genome sequencing, holds promise for rapid and precise identification of resistance genes and virulence factors, enabling tailored treatment regimens. These tools are vital for distinguishing species within the Smc and improving diagnostic accuracy.

Prevention is equally critical, particularly in healthcare settings where *S. sepilia* thrives. Strengthening infection control measures, such as rigorous sterilization protocols for medical devices and enhanced hospital hygiene practices, is imperative to limit the spread of MDR bacteria. Additionally, robust surveillance systems are needed to monitor the global prevalence and resistance patterns of *S. sepilia*, providing valuable data to inform public health interventions.

On the therapeutic front, there is a growing emphasis on exploring alternatives to conventional antibiotics. Combination therapies targeting biofilm formation and resistance mechanisms, as well as adjunctive treatments such as bacteriophage therapy, are under investigation. Bacteriophages, which specifically target bacteria, present a promising avenue for combating biofilm-associated infections and MDR pathogens like *S. sepilia*. Furthermore, research into quorum-sensing inhibitors, which disrupt bacterial communication and biofilm formation, is ongoing and may yield innovative therapeutic solutions.

To curb the rising resistance of *S. sepilia* to first-line antibiotics, antimicrobial stewardship practices must be reevaluated. Reducing the misuse of broad-spectrum antibiotics and prioritizing susceptibility-guided therapy can help mitigate selective pressures that drive resistance. Concurrently, investments in research and development for novel antibiotics, particularly those effective against biofilm-forming and MDR pathogens, remain a cornerstone of public health efforts.

The increasing clinical and geographical prevalence of S. *sepilia* highlights its potential to become a significant global health challenge. Addressing this issue requires collaborative efforts between microbiologists, clinicians, public health experts, and policymakers. These efforts should focus on enhancing diagnostic capabilities, optimizing therapeutic strategies, and strengthening infection control measures to mitigate the risks posed by this emerging pathogen. Such initiatives will not only improve our ability to manage infections caused by *S. sepilia* but also bolster broader efforts to combat antimicrobial resistance.

Taxonomy

The taxonomic history of *S. maltophilia* reflects its complex and dynamic evolutionary trajectory, shaped by advancements in molecular biology and microbial classification techniques. Initially described as *Bacterium bookeri* in 1943,[30] this bacterium was later reclassified as *Pseudomonas maltophilia* in 1961[31] due to its phenotypic resemblance to other members of the genus *Pseudomonas*. However, as scientific tools and methodologies evolved, deeper genetic analyses, including DNA–DNA hybridization, rRNA sequencing, and multilocus sequence typing (MLST), revealed its distinct genetic identity, necessitating a taxonomic revision.

In 1983, *Pseudomonas maltophilia* was reassigned to the genus *Xanthomonas*,[32] marking a significant step in its taxonomic refinement. Yet, further molecular analyses, particularly 16S rRNA sequencing, emphasized the necessity for a separate genus to accommodate this unique lineage. Consequently, the genus *Stenotrophomonas* was established in 1993 to accurately reflect its genetic and phenotypic distinctiveness. This reclassification cemented its position as a unique member of the family Lysobacteraceae, a testament to the pivotal role of molecular tools in the modern taxonomy of bacteria.

The genus *Stenotrophomonas* is composed of Gram-negative, aerobic bacteria renowned for their metabolic versatility and natural adaptability. Members of this genus are ubiquitous in diverse environments, including soil, water, plant rhizospheres, and animal hosts, underscoring their ecological significance. This adaptability, coupled with their ability to utilize a wide array of substrates, highlights their crucial role in both environmental and medical microbiology.

To date, 25 species have been validated within the genus *Stenotrophomonas*, as documented by the List of Prokaryotic Names with Standing in Nomenclature (LPSN, https://lpsn.dsmz.de/genus/Stenotrophomonas), now being referred to as Smc. First described in our study, among these species, *S. maltophilia* emerges as a prominent opportunistic pathogen, distinguished by its capacity to cause nosocomial infections, particularly in immunocompromised individuals. Its pathogenicity is largely attributed to its intrinsic resistance to multiple antibiotics, ability to form robust biofilms, and its remarkable resilience in diverse environmental and clinical settings.

This taxonomic journey from *B. bookeri* to *S. maltophilia* reflects the intricate and evolving understanding of microbial diversity. It underscores the importance of integrating phenotypic observations with molecular and genomic insights to achieve accurate and meaningful classification. As the genus continues to expand with newly validated species, it remains a focal point of research, bridging the domains of environmental microbiology and clinical infectious diseases.

Clinical Spectrum

The genus *Stenotrophomonas* is predominantly regarded as a group of environmental bacteria, with the majority of its species being nonpathogenic to humans. Historically, the notable exception within this genus has been *S. maltophilia*, a significant opportunistic pathogen recognized for its role in nosocomial infections. However, recent findings have introduced a new member to the list of pathogenic species within the Smc—*S. sepilia*. *S. sepilia* represents a potentially greater clinical threat due to its enhanced biofilm-forming capabilities and MDR profile.[28]

Stenotrophomonas sepilia has been identified and isolated from samples previously classified as *S. maltophilia*. Given their similar spectrum of characteristics, *S. sepilia* can be recovered from clinical sources commonly associated with *S. maltophilia*.

In healthcare settings, *Stenotrophomonas* has been identified as a pathogen frequently associated with contaminated medical and environmental sources. These include suction systems in dental chair units, contaminated endoscopes, tap water, central venous catheters (CVCs), and hemodialysis machines. The ability of *Stenotrophomonas* to persist in such environments highlights its resilience and adaptability. Key infection types caused by this bacterium include bacteremia, septicemia, meningitis, and soft tissue infections, with immunocompromised patients being particularly vulnerable.

Central venous catheter-related and respiratory infections are among the most common clinical manifestations of *Stenotrophomonas*. Biofilm formation on catheter surfaces not only facilitates bacterial persistence but also complicates treatment, often necessitating the removal of the catheter in addition to targeted antibiotic therapy. Hematopoietic stem cell transplant (HSCT) and bone marrow transplant (BMT) recipients are especially susceptible to *Stenotrophomonas*-related infections, primarily due to prolonged neutropenia and breaches in mucocutaneous barriers. These infections are characterized by high morbidity, underscoring the importance of rigorous infection control measures.

Community-acquired infections, while less common, further expand the clinical spectrum of *Stenotrophomonas*. Such infections may include respiratory tract infections, cellulitis, and wound infections. They often occur in individuals with underlying conditions such as chronic obstructive pulmonary disease (COPD), prior antibiotic use, or immunosuppression. The presence of *Stenotrophomonas* in community settings, particularly in water distribution systems and household environments, represents a significant risk for immunocompromised individuals.

Environmental contamination remains a key factor in the transmission of *Stenotrophomonas*. Studies have demonstrated the bacterium's presence in hospital water systems, household tap water, and other sources such as sink drains and sponges. This emphasizes the critical need for stringent water quality control and preventive measures, such as the implementation of point-of-use (POU) water filtration systems. Such interventions have been shown to significantly reduce the incidence of healthcare-associated Gram-negative bacterial infections.

Lab Diagnosis

The identification of *S. sepilia* (strain SM16975) employed a comprehensive and multidisciplinary approach, integrating phenotypic, chemotaxonomic, biochemical, metabolic, genomic, and molecular analyses. This robust methodology firmly established *S. sepilia* as a novel and distinct member of the Smc.

Phenotypic characterization provided critical insights into the strain's morphology and biochemical traits. The colonies exhibited no difference when compared to *S. maltophilia* and large, smooth, convex, and circular features, with a distinctive lavender-green colonies on blood agar and colorless appearance on MacConkey agar, consistent with nonlactose-fermenting organisms were observed.[33] Microscopically, *S. sepilia* was identified as Gram-negative, rod-shaped, and motile. Biochemical testing revealed catalase positivity and oxidase negativity, hallmarks of the genus. Antibiotic susceptibility testing, conducted using the Kirby–Bauer method (CLSI M100, 2020), demonstrated the strain's sensitivity to co-trimoxazole, levofloxacin, and minocycline, while resistance to ceftazidime was observed, a trait common to MDR members of the Smc.[28]

Chemotaxonomic profiling further reinforced the uniqueness of *S. sepilia*. Fatty acid analysis revealed a distinctive composition, including elevated levels of iso-C15:0 and anteiso-C15:0, high proportions of iso-C17:0 and iso-C13:0 3-OH, and characteristic summed features 3 and 8, comprising 16:1 ω7c/16:1 ω6c and 18:1 ω7c, respectively. The reduced presence of C16:1 ω9c added another layer of differentiation from other Smc species, cementing its distinct chemotaxonomic signature.

The strain's biochemical and metabolic capabilities were explored using the Biolog GEN III MicroPlate™ system, which demonstrated its metabolic versatility. *S. sepilia* exhibited the ability to utilize a range of substrates, including dextrin, D-maltose, D-cellobiose, and citric acid. The strain also displayed resistance to rifamycin SV, troleandomycin, lincomycin, vancomycin, and aztreonam, consistent with resistance profiles observed in other Smc members. Unique metabolic traits further distinguished SM16975 as a novel entity within the complex.

Genomic analysis provided definitive evidence of the strain's novelty. The genome of *S. sepilia* spans 4.58 megabases with

a G + C content of 66.4%. Phylogenetic analyses confirmed its placement within the Smc, highlighting its genetic distinction from other complex members. These genomic insights reinforced the findings from phenotypic, chemotaxonomic, and metabolic characterizations, providing a holistic view of the strain's unique attributes.

Molecular identification using PCR-based techniques added precision to the identification process. Specific primers targeting unique sequences in the *S. sepilia* genome were employed in a validated PCR protocol. This approach ensured high specificity and accuracy, further substantiating the classification of *S. sepilia* as a distinct species within the Smc.

This integrative identification framework showcases the distinctive phenotypic, chemotaxonomic, biochemical, metabolic, genomic, and molecular features of *S. sepilia*. These findings not only confirm its classification as a novel species but also underscore its significance within the Smc, contributing valuable insights to clinical microbiology and taxonomy.

Genomic Profiling and Identification

The genomic analysis of *S. sepilia* highlights its unique position within the Smc. Utilizing advanced taxonomic tools such as ANI, digital DNA–DNA hybridization (dDDH), and phylogenetic analyses, the study confirmed the novelty of this species while identifying key antibiotic resistance genes (ARGs), biofilm-related pathways, and virulence factors that emphasizes its clinical and epidemiological relevance.[28]

The genome of *S. sepilia* (strain SM16975), comprising 45,82,512 base pairs with a G + C content of 66.4% (NCBI accession: LXXZ00000000),[34] underwent comprehensive phylogenetic analysis. The 16S rRNA gene revealed sequence identities of 99.73, 99.58, and 99.52% with *S. pavanii*, *Pseudoxanthomonas beteli*, and *S. maltophilia*, respectively. Core genome analyses positioned SM16975 as a distinct lineage within the Smc. Further validation using tools such as JSpecies,[35] OrthoANI,[36] Compare (donovan-h-parks/CompareM), and the GGDC server confirmed ANI,[37] OrthoANI, AAI, and dDDH values below the established species delineation thresholds, collectively solidifying the classification of *S. sepilia* as a novel species.

The nomenclature of *S. sepilia*, derived from "sepsis," underscores its significant clinical implications. Recognized as the third formally described clinical species within the genus *Stenotrophomonas*—following *S. maltophilia* and S. *africana*—*S. sepilia* occupies a monophyletic clade alongside other Smc members, including *S. maltophilia*, *S. africana*, *S. pavanii*, *Pseudomonas hibiscicola*, and *Stenotrophomonas geniculata*. These species exhibit shared genetic and phenotypic traits, reinforcing their relatedness within the complex.

A pivotal outcome of this genomic analysis was the development of a highly specific PCR assay designed to accurately differentiate *S. sepilia* from other Smc members. The assay targets a unique sequence within the *S. sepilia* genome, employing the following primers—forward primer: 5′-GTTCTCGTTGCTGGATGATGCG-3′ and reverse primer: 5′-AGTCCGTTACCGTCTTTGATCG-3′. The PCR protocol includes an initial denaturation at 95°C for 3 minutes, followed by 30 cycles of denaturation (95°C for 1 minute), annealing (54°C for 30 seconds), and extension (72°C for 1 minute), with a final extension step at 72°C for 10 minutes. The specificity of the assay was validated using SM16975 as the positive control and S. *maltophilia* ATCC 13637 as the negative control. Visualization on a 2% agarose gel confirmed species-specific amplification, with bands appearing exclusively for the positive control.

The integration of genomic profiling with PCR-based molecular tools provides a robust framework for the rapid and precise identification of *S. sepilia*. This methodology advances diagnostic microbiology and epidemiological studies by enabling accurate differentiation of *S. sepilia* from other Smc members. These findings highlight the clinical significance of *S. sepilia*, particularly in the context of nosocomial infections, and emphasize the need for ongoing research into its pathogenic mechanisms and resistance traits.

Antimicrobial Susceptibility Testing

Understanding the antimicrobial susceptibility of *S. sepilia*, a clinically significant member of the Smc, is critical for optimizing treatment strategies. Comprehensive antimicrobial susceptibility testing (AST) has provided valuable insights into the resistance profiles and therapeutic options for this MDR pathogen. Minimum inhibitory concentration (MIC) testing using microbroth dilution (MBD) and E-test methods revealed key findings, positioning levonadifloxacin as a promising alternative therapy. MICs for levonadifloxacin and levofloxacin were determined following Clinical and Laboratory Standards Institute (CLSI) guidelines, utilizing Ezy MIC™ strips (0.002–32 mg/L) to ensure precision. Quality control with *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 confirmed the reliability of results, which adhered to CLSI standards. Other antibiotics evaluated included minocycline, chloramphenicol, ceftazidime, and co-trimoxazole. In the absence of CLSI-specific guidelines for *Stenotrophomonas*, levofloxacin breakpoints were applied to levonadifloxacin.[29]

Stenotrophomonas sepilia employs diverse antimicrobial resistance mechanisms, making it a formidable nosocomial pathogen. Genomic analysis identified 46 ARGs across seven classes, including resistance-nodulation-division (RND) efflux pumps, beta-lactamases, and aminoglycoside phosphotransferases (APH). Prevalent aminoglycoside acetyltransferase (AAC) genes were observed, while aminoglycoside nucleotidyltransferases (ANT) genes were absent. Multidrug efflux systems, such as the SmeABC efflux pump, play a central role in resistance. Interestingly, some Smc strains, including genospecies 1 and 7, lack the SmeABC pump, resulting in reduced resistance to aminoglycosides, beta-lactams, and fluoroquinolones. For sulfonamide resistance, the *sul1* and *sul2* genes were detected in only 3.6% of clinical isolates, preserving low resistance to trimethoprim-sulfamethoxazole, which remains widely considered first-line treatment for *S. sepilia* infections. These findings highlight *S. sepilia*'s adaptability to antimicrobial pressure while identifying potential therapeutic targets for effective management.

Resistance mechanisms in *S. sepilia* also include efflux pumps and beta-lactamases, which contribute to its MDR profile. Certain strains exhibited notable resistance to ceftazidime and cefepime, highlighting the limitations of these agents against *S. sepilia*. Conversely, minocycline and trimethoprim-sulfamethoxazole demonstrated high efficacy, with the former showing universal susceptibility and the latter effective against 98% of isolates. Prevalence studies conducted at Postgraduate Institute of Medical Education and Research, Chandigarh, revealed that among 116 clinical *S. maltophilia* isolates identified *via* MALDI-TOF MS, 46 were confirmed as *S. sepilia* through PCR, representing 40% of isolates. These findings accentuates the clinical importance of this pathogen and the need for tailored therapeutic approaches.

Antimicrobial susceptibility testing further highlighted diverse resistance and susceptibility patterns. Resistance to ceftazidime was observed in 59% of isolates, and chloramphenicol showed limited efficacy with a resistance rate of 63%. However, levofloxacin inhibited 98% of isolates, with MICs ranging from 0.25 to 2 mg/L. One isolate exhibited significant levofloxacin resistance, with an elevated MIC of 16 mg/L. Levonadifloxacin demonstrated comparable efficacy to levofloxacin, inhibiting 98% of isolates but with consistently lower MICs (0.25–1 mg/L), signifying greater *in vitro* potency. Notably, the levofloxacin-resistant isolate displayed intermediate susceptibility to levonadifloxacin, with an MIC of 6 mg/L by E-test and 4 mg/L by MBD, suggesting its potential as an alternative therapy for levofloxacin-resistant infections.

Levonadifloxacin, a next-generation fluoroquinolone, emerged as a particularly potent option, with lower MICs compared to levofloxacin across isolates. Its superior *in vitro* potency positions it as a viable alternative for treating infections caused by *S. sepilia*, especially those resistant to standard fluoroquinolones. Minocycline consistently demonstrated the highest efficacy among the tested antibiotics, maintaining universal activity across all isolates. Co-trimoxazole, levofloxacin, and minocycline also exhibited high efficacy, further emphasizing their roles as cornerstone treatments for *S. sepilia* infections.

These findings underline the clinical potential of levonadifloxacin, particularly for addressing MDR infections. However, further validation through clinical breakpoints and pharmacokinetic/pharmacodynamic (PK/PD) studies is necessary to establish its definitive therapeutic utility. This comprehensive AST profile highlights the importance of continuous research and clinical vigilance in managing *S. sepilia* infections, providing a foundation for evidence-based treatment strategies tailored to this emerging nosocomial pathogen.

Epidemiology

The epidemiology of *S. sepilia*, a clinically significant member of the Smc, highlights its emergence as a major nosocomial pathogen with varied regional and global prevalence. Its ability to thrive in healthcare environments and its association with invasive infections reinforces its clinical relevance. Recent surveillance and genomic studies have revealed its expanding presence across continents, with a significant impact in both developed and developing healthcare systems. The pathogen's high prevalence in hospital settings and its MDR profile emphasize the need for targeted infection control measures and therapeutic strategies.

Indian Scenario

In India, *S. sepilia* has been identified as a significant contributor to nosocomial infections. Among clinical isolates studied, *S. sepilia* accounted for 40% of Smc-related infections at Postgraduate Institute of Medical Education and Research, Chandigarh,[29] highlighting its prevalence in healthcare environments. Data from other centers across the country further illustrate its widespread presence: *S. sepilia* constituted 31% of Smc isolates at Tata Memorial Hospital (TMH), Mumbai; 43% at Apollo Hospital, Bhubaneshwar; 50% at Kailash Hospital (KH), Noida; and 100% at Mahatma Gandhi Medical College (MGMC), Jaipur. However, it is noteworthy that the MGMC data is based on only a single sample, which limits broader generalization from this result. These findings emphasize the adaptability and clinical significance of *S. sepilia* in diverse healthcare settings across the country.

Global Scenario

Globally, *S. sepilia* exhibits varied prevalence patterns influenced by regional healthcare practices, antibiotic usage, and environmental factors. Africa reports the highest prevalence, where *S. sepilia* dominates Smc isolates, emphasizing its clinical importance in the region.[38] In Asia, it accounts for 19% of Smc isolates, reflecting its growing role as a nosocomial pathogen. In Europe, *S. sepilia* constitutes 14.3% of Smc isolates, ranking second to *S. maltophilia* (51.8%) and surpassing *S. geniculata* (4%). North America reports a lower prevalence of 2.3%, with *S. muris* (14.8%) being more common. In contrast, South America and Oceania continue to report S. *maltophilia* as the predominant species.

A comprehensive genomic analysis of 734 isolates worldwide identified *S. sepilia* as the second most prevalent strain within the Smc, with increasing clinical relevance in Asia, Europe, and North America. These global findings highlight the pathogen's expanding footprint and its role as an emerging threat in hospital settings. Regional differences in prevalence reflect variations in healthcare infrastructure, antibiotic stewardship, and infection control practices, underscoring the need for tailored interventions.

Sample source data further highlight the clinical significance of *S. sepilia*. Among 471 Smc strains studied, 64.2% were isolated from clinical environments, demonstrating its association with invasive infections. These infections frequently involve *S. sepilia*, alongside other pathogenic genospecies such as genospecies 3, 4, and 5. In contrast, genospecies 18, 20, and 21 were exclusively isolated from nonclinical environments, underscoring the ecological versatility of Smc species. This distinction between invasive and environmental strains reinforces the clinical importance of *S. sepilia* in hospital settings.

The epidemiological data underline the need for robust surveillance systems, region-specific infection control strategies, and effective therapeutic options to address the growing challenge posed by *S. sepilia*. Its evolving prevalence and resistance profile highlight the necessity of ongoing research and multidisciplinary efforts to mitigate its impact on public health.

Virulence of *Stenotrophomonas sepilia*

The virulence of *S. sepilia* is underpinned by multiple factors that enhance tissue invasion and environmental persistence, making it a formidable nosocomial pathogen. Like its other species, S. *maltophilia*, *S. sepilia* exhibits robust biofilm formation, efflux systems, and enzymatic activity, which collectively drive its pathogenicity. Among 13 species within the Smc, *S. sepilia* demonstrated the highest swimming motility, facilitating efficient tissue invasion and biofilm formation comparable to that of S. *maltophilia*. In contrast, genospecies 3 displayed the weakest biofilm-forming ability, further underscoring *S. sepilia*'s virulent potential.[28]

Stenotrophomonas sepilia's strong biofilm-forming ability significantly complicates treatment in clinical settings. This trait contributes to its persistence on medical devices, host tissues, and its association with chronic infections. Such persistence is especially problematic in nonhealing wounds, such as diabetic foot ulcers, where biofilm-forming pathogens demonstrate resistance to antimicrobial therapies. The presence of biofilm acts as a physical and metabolic barrier, shielding bacteria from antibiotics and immune responses, thereby prolonging infections and increasing treatment challenges.

These virulence traits highlight the necessity for innovative therapeutic strategies targeting both biofilm disruption and resistance mechanisms. Promising findings involving a novel natural formulation, VG111 (unpublished data), indicate potential breakthroughs in addressing the clinical challenges posed by S. *sepilia*. This formulation reportedly exhibits activity against biofilm-associated pathogens, offering hope for improved management of recalcitrant infections.

The combined resistance and virulence traits of *S. sepilia*—spanning biofilm formation, tissue invasion, and environmental persistence—accentuates the complexity of managing infections caused by this emerging pathogen. These challenges necessitate a dual focus on disrupting biofilms and countering antimicrobial resistance, paving the way for novel avenues in therapeutic intervention and infection control.

Pathogenicity Assessment in *Caenorhabditis elegans* Model (Unpublished Data)

In *Caenorhabditis elegans* killing assays, *S. sepilia* SM16975 exhibited notable pathogenicity, leading to complete nematode mortality within approximately 216 hours. This killing rate was slightly faster compared to *S. maltophilia* ATCC13637, which achieved total mortality in around 230 hours. As a reference pathogenic control, the virulent strain *P. aeruginosa* PA14 induced nematode death within a significantly shorter time frame of 80 hours. In contrast, the nonpathogenic *E. coli* OP50, commonly used as a laboratory food source and negative control, allowed approximately 40% of the nematodes to survive beyond 230 hours, with the surviving population maintaining good motility. However, the *C. elegans* pmk-1 (km25) mutation resulted in increased susceptibility to E. *coli* OP50, with all mutants perishing by 216 hours. This finding highlights the importance of the p38-MAPK pathway in baseline resistance to nonpathogenic bacteria.

Interestingly, *C. elegans* pmk-1 (km25) mutants, which harbor a mutation in the p38-MAPK pathway, exhibited no significant difference in susceptibility to *S. sepilia* infection when compared to wild-type worms. The time to death in the mutants remained consistent with that observed in wild-type nematodes, approximately 218 hours, suggesting that the p38-MAPK pathway does not play a critical role in the nematode's defense against S. *sepilia*.

Stenotrophomonas sepilia as a Promoter in Farming

Stenotrophomonas sepilia ZH16, which was isolated from the internal tissues of rice, exhibits noteworthy capabilities as a plant-growth-promoting bacterium (PGPB). These include the solubilization of phosphate and zinc, two nutrients that are highly essential for robust crop development, and the production of the plant hormone IAA. Such metabolic traits combine to enhance nutrient availability to the plant host while also stimulating root and shoot growth. In greenhouse experiments, *S. sepilia* ZH16 demonstrated significant improvements in overall rice seedling growth parameters, thereby validating its potential as beneficial in agricultural systems.[27]

Extensive genomic analysis offers further clarity on how S. *sepilia* ZH16 exerts these plant-beneficial effects. The bacterium's genome consists of genes responsible for IAA biosynthesis, nutrient solubilization (including an operon for the cofactor pyrroloquinoline quinone, crucial for phosphate release), and biosynthesis of exopolysaccharides (EPS) involved in root adherence and stress protection. Moreover, the presence of multiple antioxidant defenses, most notably pathways for glutathione and ergothioneine synthesis, explains *S. sepilia* ZH16's strong tolerance to oxidative stress. This robust oxidative stress response suggests its suitability for challenging agricultural environments, including soils afflicted by high metal concentrations.

Studies also show that *S. sepilia* ZH16 displays mild antifungal properties against *Pyricularia oryzae*, the pathogen responsible for rice blast, indicating potential dual benefits as both a biofertilizer and a biocontrol agent. Meanwhile, safety assessments reveal a lack of harmful virulence traits commonly associated with pathogenic *S. maltophilia* strains. Therefore, the strain's beneficial traits—root-colonizing capacity, hormone production, nutrient solubilization, and tolerance to stress—make *S. sepilia* ZH16 an ideal candidate for eco-friendly, sustainable agriculture practices, particularly in rice-farming systems prone to stressors like poor soil conditions or pathogen pressure. This strain emerges as a promising resource for the development of advanced biofertilizer formulations, potentially reducing reliance on chemical inputs and enhancing resilience in modern farming systems.

Conclusion

Stenotrophomonas sepilia has been unequivocally established as a novel and clinically significant member of the Smc through a multidisciplinary approach that encompasses phenotypic, chemotaxonomic, biochemical, genomic, and PCR-based methodologies. These exhaustive analyses underscore its unique taxonomy, genomic composition, and metabolic capabilities, culminating in the development of a species-specific PCR assay that addresses critical needs in both clinical and environmental microbiology.

Globally, *S. sepilia* demonstrates considerable prevalence, with notable dominance reported in Africa and substantial rates of occurrence in Asia and Europe. The pathogen's prominence in India, in particular, highlights its adaptability and clinical impact, especially within healthcare facilities. As an opportunistic pathogen, its MDR profile, reinforced by strong biofilm formation and heightened swimming motility, poses significant risks in hospital environments and among immunocompromised patient populations.

Genomic investigations have shed light on the multiple resistance mechanisms of *S. sepilia*, including RND efflux pumps, beta-lactamases, and aminoglycoside-modifying enzymes such as APH and AAC. The absence of ANT genes and low rates of sulfonamide resistance genes (*sul1*, *sul2*) preserve the bacterium's susceptibility to trimethoprim-sulfamethoxazole, which remains an important first-line therapy. Minocycline consistently exhibits universal efficacy, and the next-generation fluoroquinolone levonadifloxacin displays lower MIC values than levofloxacin, highlighting its promise in clinical management. However, further clinical trials and PK/PD studies are warranted to validate levonadifloxacin's therapeutic potential.

In addition to its clinical importance, there is growing recognition of *S. sepilia* strains that exhibit beneficial traits in agricultural contexts. Certain isolates, such as *S. sepilia* ZH16, have demonstrated robust plant-growth-promoting attributes, including phosphate and zinc solubilization and the production of phytohormones (e.g., IAA). These properties bring out a fascinating dual identity: while one facet of *S. sepilia* presents tangible risks as an emerging nosocomial pathogen, another shows potential utility in sustainable agriculture and crop yield enhancement.

Nonetheless, the strong biofilm-forming capacity of *S. sepilia* and its notable motility make persistent colonization on medical devices and within host tissues a challenging reality. In chronic infections such as diabetic foot ulcers, biofilm-related resistance can significantly diminish the success of antimicrobial regimens. Innovative approaches, including therapies aimed at disrupting biofilm formation and motility, are critical for overcoming these obstacles. Early research on formulations like VG111 suggests that novel agents with anti-biofilm activity may offer new avenues for intervention.

Moving forward, a comprehensive understanding of both the clinical and ecological dimensions of *S. sepilia* will be pivotal. Future investigations should expand on the environmental and agricultural roles of this bacterium, especially for harnessing its plant-growth-promoting mechanisms in farming. Concurrently, ongoing genomic and epidemiological studies must inform more targeted diagnostic tools, combination treatments, and refined infection control measures to confront the pathogen's MDR profile. In this way, research can better elucidate how *S. sepilia* thrives across distinct environments—healthcare and agricultural alike—and shape multifaceted strategies for managing its dual impacts.

In sum, *S. sepilia* represents a significant new addition to the Smc, bringing both profound implications for public health and notable promise in sustainable agriculture. Bolstered by robust surveillance, continued research, and forward-looking therapeutic and environmental interventions, the global scientific and medical communities can more effectively respond to this emerging pathogen while also exploring its intriguing potential as a contributor to farming innovation.

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