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# Susceptibility of clinical isolates of novel pathogen *Stenotrophomonas sepilia* to novel benzoquinolizine fluoroquinolone levonadifloxacin

**Surajit Chakraborty [1], Nishant Shekhar[1], Lipika Singhal[2], Rajneesh Singh Rawat[1], Ajay Duseja[3], Rahul K. Verma[4], Kanika Bansal[5], Ivneet Kour [2], Sanjay Biswas[6], Ekadashi Rajni[7], Suneeta Sahu[8], Prabhu B. Patil [5] and Vikas Gautam [1]***

*[1]Department of Medical Microbiology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India; [2]Department of Microbiology, Government Medical College & Hospital, Chandigarh, India; [3]Department of Hepatology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India; [4]Chemical Biology Unit, Institute of Nano Science and Technology, Mohali, India; [5]Department of Microbiology, Tata Memorial Hospital, Mumbai, Maharashtra, India; [6]Bacterial Genetics, Geomics and Evolution Laboratory, CSIR-Institute of Microbial Technology, Chandigarh, India; [7]Department of Microbiology, Mahatma Gandhi Medical College & Hospital, Jaipur, India; [8]Department of Clinical Microbiology & Immunoserology, Apollo Hospital, Bhubaneswar, Odisha, India*

*Corresponding author. E-mail: r_vg@yahoo.co.uk

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**Background:** *Stenotrophomonas sepilia*, identified in 2021, is part of the *Stenotrophomonas maltophilia* complex (Smc) and shares high genomic identity with *S. maltophilia*. Resistance to levofloxacin, the recommended fluoroquinolone for *S. maltophilia*, is being increasingly reported. Recent studies indicate that levonadifloxacin, a novel benzoquinolizine, may be more effective. This study evaluates the antimicrobial efficacy of levofloxacin and levonadifloxacin against clinical isolates of *S. sepilia*.

**Objectives:** To assess the antibacterial effectiveness of levofloxacin and levonadifloxacin against novel pathogen *S. sepilia.*

**Methods:** A total of 116 *S. maltophilia* isolates, identified by MALDI-TOF MS, were collected from five centres across India. *S. sepilia* was confirmed by PCR using primers targeting a unique genomic sequence (NCBI accession number LXXZ00000000.1). Minimum inhibitory concentrations (MICs) of levonadifloxacin and levofloxacin were determined by using the microbroth-dilution method and Etest as per CLSI guidelines. The levofloxacin breakpoint was used to interpret MICs of levonadifloxacin.

**Results:** Among a total of 116 circulating *S. maltophilia* isolates collected, 46 were identified as *S. sepilia*, representing a prevalence rate of (~40%), thus highlighting its significance as an important pathogen within the Smc. Both levofloxacin and levonadifloxacin demonstrated a 98% inhibition rate against the 46 *S. sepilia* tested. Only one *S. sepilia* isolate resistant to levofloxacin showed intermediate susceptibility to levonadifloxacin, which consistently had lower MICs.

**Conclusions:** Levofloxacin and levonadifloxacin show similar susceptibility rates against *S. sepilia*, with levonadifloxacin exhibiting lower MICs. Further studies are required to establish clinical utility of levonadifloxacin in managing these infections.

## Introduction

*Stenotrophomonas sepilia* is a novel bacterial species and the newest member of the *Stenotrophomonas maltophilia* complex (Smc). In 2021, our group discovered and reported the novel bacterial pathogen after its isolation from the blood specimen of an immunocompetent individual undergoing cardiothoracic vascular surgery in the tertiary care hospital of our institute (PGIMER, Chandigarh, India).[1] Following its discovery, *S. sepilia* is now substantiated as a prevalent pathogen of Smc complex

as mentioned in the recent study by Li *et al*. (2024).[2] According to their findings, *S. sepilia* has emerged as the most common strain within the Smc in Africa and holds the position of the second most prevalent strain globally since its identification. The worldwide context in which *S. sepilia* emerges is one of growing concern, as *S. maltophilia* is known for its complex antimicrobial resistance mechanisms. These mechanisms pose significant therapeutic challenges, especially in hospital settings where multidrug-resistant strains have been documented.[3–5] Recent surveillance studies indicate a rising trend in resistance to commonly used antibiotics, with an emphasis on the critical importance of monitoring and reporting resistance patterns.[6,7] This broader pattern of resistance emphasizes the necessity of research into the antibiotic susceptibility of newly identified pathogens within the Smc, such as *S. sepilia.* Understanding the antimicrobial susceptibility of *S. sepilia* is crucial for optimal antibiotic selection, which minimizes antimicrobial resistance and prevents toxicity from extended or intensive antibiotic use. Moreover, finding and assessing newer agents to counter a new pathogen efficiently may prove advantageous in a future scenario of a possible emergence of resistance.

Levonadifloxacin is a newer benzoquinolizine (a new subclass of fluoroquinolone, $C_{19}H_{21}FN_2O_4$) with a broad-spectrum antimicrobial activity. A worldwide upsurge in levofloxacin resistance in isolates of *S. maltophilia* is evident in recent reports.[8,9] The spectrum of antimicrobial activity of levonadifloxacin covers a range of both Gram-positive and -negative pathogens including some anaerobes.[10] Among the aforementioned, the most extensively studied and demonstrated antibacterial activity is against the 'difficult to treat' MRSA and hetero-vancomycin intermediate *S. aureus* (h-VISA).[11] Levonadifloxacin also demonstrates activity against variety of Gram-negative organisms. With high susceptibility rates observed against *Acinetobacter baumannii* (98%) and *Pseudomonas aeruginosa* (97%), levonadifloxacin has also demonstrated high susceptibility against *S. maltophilia* with 36/38 (∼95%) isolates being susceptible at ≤2 mg/L whereas two isolates are intermediate sensitive at 4 mg/L.[12,13]

Given the increasing clinical challenges posed by opportunistic pathogens and the rising trends of antimicrobial resistance, the recommendation of fluoroquinolones has become more selective. In this milieu, newer antimicrobial agents such as levonadifloxacin are gaining attention for their potential to treat resistant infections effectively.[14] It is against this backdrop of necessity and innovation that our study is positioned; we aim to compare the antimicrobial efficacy of the established levofloxacin and the novel levonadifloxacin against clinical isolates of *S. sepilia.* Our findings could have significant implications for the management of infections caused by this opportunistic pathogen.

## Material and methods

### *Collection of* S. maltophilia *isolates*

A total of 116 clinical isolates of *S. maltophilia* (non-duplicate) were collected consecutively between December 2021 and October 2022 from five different centres across India: PGIMER, Chandigarh (70 isolates), Tata Memorial Hospital, Mumbai (32 isolates), Apollo Hospital, Bhubaneshwar, Odisha (seven isolates), Kailash Hospital, Noida, Uttar Pradesh (six isolates) and Mahatma Gandhi Medical College, Jaipur, Rajasthan (one isolate). These isolates were collected without any bias towards particular clinical specimens, ensuring a diverse and representative sample. All 116 *S. maltophilia* isolates were identified by in the concerned microbiology laboratories in the respective centres and sent to our research laboratory in the Department of Medical Microbiology, PGIMER, Chandigarh, India. On receipt of the isolates, the isolates were sub-cultured and the identity of the isolates was reconfirmed by MALDI-TOF MS (Bruker Daltoniks, Germany) using the freshly sub-cultured pure colonies. MALDI-TOF MS is currently unable to identify *S. sepilia* as a separate species because the spectral database does not contain the unique peptide mass fingerprints of novel species *S. sepilia*. Hence, MALDI-TOF identified the colonies of all the 116 isolates (non-fermenting Gram-negative bacilli) as *S. maltophilia*.

### *Identification of* S. sepilia *by PCR*

All 116 clinical isolates of *S. maltophilia* (MALDI-TOF MS identified) were subjected to a PCR that was devised and optimized in our laboratory for the identification of *S. sepilia*. Primers (forward 5′-GTTCTCGTTGCTGGATGATGCG-3′; reverse 5′-AGTCCGTTACCGTCTTTGATCG-3′) targeted a unique sequence present in the genome of *S. sepilia* (NCBI accession number LXXZ00000000.1).[1] The cycling conditions to amplify the target were set as follows: 3 min at 95°C for initial denaturation, 30 cycles of denaturation at 95°C for 60 s, annealing at 54°C for 30 s, extension at 72°C for 1 min and final extension at 72°C for 10 min. DNA from *S. sepila* SM16975 (=JCM32102; =KCTC62052) (accession number LXXZ00000000) and *S. maltophilia* ATCC 13637 were kept as positive and negative controls respectively for the *S. sepilia* identification PCR. Amplified PCR products were resolved in 2% agarose gel (SeaKem LE Agarose, LONZA, India).

### *Determination of minimum inhibitory concentrations*

Minimum inhibitory concentrations (MICs) of levonadifloxacin and levofloxacin were determined by using the microbroth-dilution (MBD) method and Etest following the standard CLSI guidelines.[15] Estrips (Ezy MIC™ strip, HIMEDIA, Mumbai, India) with a concentration gradient ranging from 0.002 to 32 mg/L were used. *Pseudomonas aeruginosa* ATCC 27853 (reference MIC range 0.5–4 mg/L) and *Escherichia coli* ATCC 25922 (reference MIC range 0.03–0.25 mg/L) were used as the quality control strains. MICs were determined for levofloxacin, minocycline, chloramphenicol, ceftazidime and trimethoprim-sulfamethoxazole (co-trimoxazole) using the MBD method. As there is no breakpoint prescribed by the CLSI for clinical isolates of *Stenotrophomonas* spp., breakpoints of levofloxacin were extrapolated for the interpretation of MICs of levonadifloxacin against all isolates of *S. sepilia*.[15]

## Results and discussion

The revival rate of the transported and stored isolates was 100% (116/116). Among all the 116 clinical isolates of *S. maltophilia* (identified by MALDI-TOF MS), PCR amplification yielded a product of 192 bp from the DNA of 46 isolates (including the positive

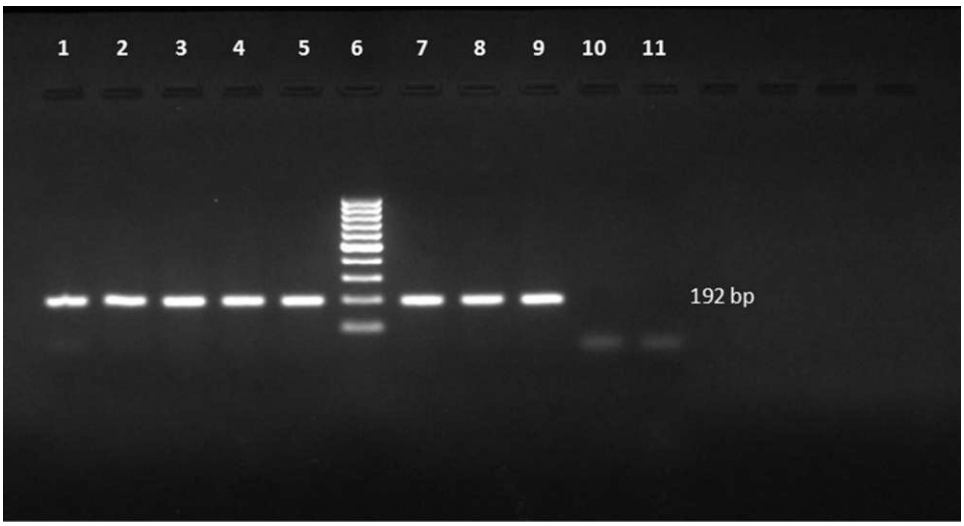


**Figure 1.** A unique target of 192 bp was amplified in all *S. sepilia* isolates, whereas no amplification of any such target was observed in the case of *S. maltophilia* (10 and 11).

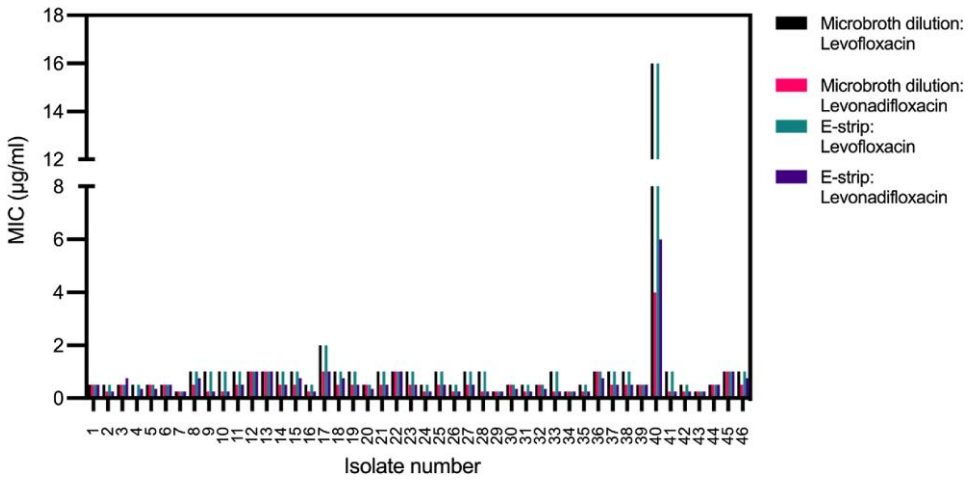


**Figure 2.** Comparison of MIC values of levofloxacin and levonadifloxacin, respectively. For most of the isolates, the MIC of levonadifloxacin was lower than that of levofloxacin. For isolate no. 40, levofloxacin showed a MIC of 16 mg/L whereas levonadifloxacin also exhibited higher value of MIC (6 mg/l) by Estrip and 4 mg/L by microbroth dilution that falls into the intermediate susceptible category as extrapolated from levofloxacin MIC breakpoints as per the CLSI guideline. A broken bar indicates a high value of MIC.

control) that were identified as *S. sepilia.* (Figure 1). We observed a prevalence of 40% (46/116) for *S. sepilia* among the pre-identified *S. maltophilia* isolates with no inclusion of duplicate isolates. The term 'prevalence' in this study refers to the proportion of *S. sepilia* identified among the circulating isolates of *S. maltophilia* collected over 1 year. Our findings indicate that 39.66% (~40%) of the circulating isolates initially identified as *S. maltophilia* were confirmed to be *S. sepilia* through PCR. The distribution of *S. sepilia* within the circulating *S. maltophilia* isolates received consecutively at PGIMER, Chandigarh alone was 41.43% (29/70). The case histories were duly collected for the associated cases further ensuring that duplicate isolates were removed from the analysis.

Among all the *S. sepilia* clinical isolates in this study, 59% and 63% were resistant to ceftazidime and chloramphenicol, respectively, which was determined using the MBD method while, 98% and 100% of the isolates were susceptible to trimethoprim-sulfamethoxazole and minocycline, respectively (data not shown).

Levofloxacin exhibited 98% inhibition rate (45 out of 46 isolates) of *S. sepilia*, displaying MIC values between 0.25 and 2 mg/L (Figure 2). Only a single isolate of *S. sepilia* demonstrated resistance to levofloxacin, with a MIC recorded at 16 mg/L in both the Etest and MBD methods. Similarly, levonadifloxacin inhibited the same percentage of *S. sepilia* isolates (98%, 45/46) with MICs ranging from 0.25 to 1 mg/L. Notably, the isolate resistant to

levofloxacin was found to have intermediate susceptibility to levonadifloxacin, with a MIC of 6 mg/L on the Estrip and 4 mg/L as per the MBD results, detailed in Supplementary Figures S1 and S2 (available as Supplementary data at *JAC-AMR* Online).

According to our study *S. sepilia* represents a significant proportion of ∼40% of *S. maltophilia* circulating cases. Furthermore, it has been established as the second most common strain within the Smc globally, with a notably high prevalence in Africa, while being the second most common in Europe (14.3%) and Asia (19%) as determined by a comprehensive genomic analysis of 734 genomes by Li *et al.*[2] Amid the range of antibiotics evaluated, minocycline leads in effectiveness against *S. sepilia*, demonstrating the highest susceptibility rate among the tested isolates. Trimethoprim-sulfamethoxazole and levofloxacin also show high susceptibility rates and are part of the advised treatment protocol. Despite similar susceptibility rates between levofloxacin and levonadifloxacin for *S. sepilia* isolates, the lower MIC values observed for levonadifloxacin indicate its potential for greater effectiveness. However, lower MIC values alone do not imply superior efficacy without considering drug exposure (pharmacokinetic/pharmacodynamics targets) and established clinical breakpoints. Therefore, further studies are necessary to confirm the clinical utility of levonadifloxacin in managing these infections.

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This study was carried out as part of our routine work.

## Transparency declarations

None to declare.

### *Author contributions*

Surajit Chakraborty: Investigation, methodology, original draft preparation, data curation. Nishant Shekhar: Methodology, formal analysis, review and editing. Lipika Singhal: Supervision, bacterial samples, investigation. Rajneesh Singh Rawat: Methodology, formal analysis. Ajay Duseja: Conceptualization, supervision. Rahul K. Verma: Conceptualization, supervision. Kanika Bansal: Methodology, formal analysis. Ivneet Kour: Supervision, bacterial samples. Sanjay Biswas: Supervision, bacterial samples. Ekadashi Rajni: Supervision, bacterial samples. Suneeta Sahu: Supervision, bacterial samples. Prabhu B. Patil (PBP)*: Conceptualization, supervision, review and editing. Vikas Gautam (VG)*: Project administration, conceptualization, supervision, investigation, review and editing.

## Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC-AMR* Online.

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