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Review Article

Stenotrophomonas maltophilia: An Overlooked Enemy Disguised as a Friend

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Abstract

Stenotrophomonas maltophilia is one of the most common emerging nosocomial pathogenic non-fermenting Gram-negative bacilli (NFGNBs). The genus *Stenotrophomonas* comprises 19 validly published species. The bacteria in the genus are environmental commensals, especially in close association with plants. They are auspicious agents for biotechnological applications in agriculture. *Stenotrophomonas maltophilia* is the most predominant species known to cause disease in humans and animals. It was once considered a pathogen of low virulence but now emerged as an important nosocomial opportunistic pathogen causing pneumonia and bacteraemia in immunocompromised individuals. The plasticity to various niches and hosts of this pathogen is primarily attributed to the mutation rate and intrinsic resistance to multiple antibiotics. The advent of various diagnostic methods increased the reporting of these pathogens from different clinical and non-clinical environments in recent years. In the past two decades, this pathogen caused various clinical conditions in animals. Frequent reports of this pathogen in hospital environments, animals, and foods of animal origin, suggest their possible role as important reservoirs for human infections and challenges for the future. Here we discuss the different microbial characteristics, epidemiology, and emerging concerns of this complex group of organisms with special reference to *S. maltophilia*.

Keywords: *Stenotrophomonas maltophilia;* Multidrug Resistance; Hospital-acquired Infections; Animals; Environment, Virulence Traits; Secondary Bacterial Infections; Colonization

Introduction

Stenotrophomonas maltophilia is a non-fermenting Gram-negative bacillus (NFGNB), often isolated from soil, water, plants, humans, and animals [1]. It causes severe nosocomial infections in humans including respiratory infections and bacteraemia, furthermore, this bacterium is very versatile and has been reported to cause infection in almost every organ of humans like the eyes, liver, gastrointestinal tract, spinal cord, and urinary tract [2]. It is one of the pathogens recovered from cystic fibrosis (CF) patients [3]. It is an emerging pathogen of concern and the third most common among the NFGNB group after *Pseudomonas* and *Acinetobacter* species strains. Owning intrinsic resistance genes against a wide range of antibiotics including fluoroquinolones, carbapenems, β-lactams, aminoglycosides, chloramphenicol, macrolides, tetracycline and polymyxins highlighted it as one of the most important multidrug-resistant pathogens [4]. World Health Organization (WHO) also considered this organism as an important

Gram-negative multidrug-resistant nosocomial pathogen. *Stenotrophomonas maltophilia* strains are commensal with low virulence but deadly as an opportunistic pathogen associated with high morbidity and mortality rates among immunocompromised patients [5]. These patients are more vulnerable to infections than healthy people. Antibiotic prophylaxis or treatment is the major factor impeding infection which also provides a high antibiotic load in the clinical environment. Thus, organisms like *S. maltophilia* with high resistance have higher chances of survival and hence produce infections. The infections are seen in both forms, community-acquired, and nosocomial infections. Some outbreak investigations in intensive care units (ICU) of hospitals revealed tap water, kitchen drinking water and sinks in the ICU kitchen, ventilator-suction systems, hospital air and catheters as the possible source of infections [2]. Many reports are available as pseudo-outbreaks (occurrences of apparent higher disease frequency because of improved surveillance or another factor unrelated to the disease itself) [2]. These reports identified the bronchoscopy procedure as an important predisposing factor for human infections [2].

Stenotrophomonas maltophilia is a unique bacterium with beneficial and harmful effects (dual interactions) in the ecosystem. On one side they are emerging as multidrug-resistant opportunistic pathogens causing direct infection or secondary infections to various pathological conditions [6]; on the other side, they are used as biocontrol, bioremediation, or stress protection agents for crops [7]. To combat these pathogens, researchers are focusing to understand their epidemiology, especially their environmental interactions and molecular mechanisms of drug resistance and pathogenesis. Over the past three decades, *S. maltophilia* has become a serious human pathogen from a plant-associated species. It is reported to have a high mutation rate in the genome. The clinical strains were having hypermutation activity and environmental strains showed a broad range of mutation rates [8]. This indicated the importance of mutation rate in the emergence of pathogens in new niches and hosts. It is still difficult to discriminate between good and bad *S. maltophilia* strains [7], hence the risk of contracting the infection from contaminated food, environment and animals cannot be completely ruled out. In recent years, reports of *S. maltophilia* infections in animals have increased, suggesting them as a potential reservoir of infections for humans. In the post-COVID-19 pandemic era, the number of infections and duration of hospitalization of patients have increased, especially in ICUs. This substantially increased the risk of hospital-acquired secondary bacterial infections and colonization of *S. maltophilia* and other related pathogens [8]. The difficulty in diagnosis and treatment of this pathogen in hospitalized patients complicates the recovery. The current review addressed the various microbiological characteristics of *Stenotrophomonas* species, highlighting their occurrence in animals that could be an important reservoir.

Etymology

The term *S. maltophilia* is derived from Greek and Latin words: *Stenos*, narrow; *trophos*, the one who feeds; *monas*, a unit, monad, i.e., a unit feeding on few substrates; and *malt*, malt sugar; *philos*, friend; i.e., a friend of malt. *Stenotrophomonas maltophilia* can ferment maltose but not glucose.

Taxonomic Classification

Domain	**Bacteria**
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Xanthomonadales
Family	Xanthomonadaceae
Genus	*Stenotrophomonas* [10]
Type Species	*Stenotrophomonas maltophilia*

Table a

The genus *Stenotrophomonas* is heterogeneous both genetically and phenotypically. The characterization of different species of *stenotrophomonas* is expanding rapidly due to its importance in biotechnological applications. The 16s rRNA gene sequence is highly conserved, making its taxonomy complicated. Presently, it encompasses 20 validly published species including *S. acidaminiphila, S. africana, S. bentonitica*, *S. chelatiphaga, S. daejeonensis, S. dokdonensis, S. ginsengisoli, S. humi, S. indicatrix*, *S. koreensis, S. geniculate*, *S. nitroreductase*, *S. koreensis*, *S. lactitubi*, *S. pavanii*, *S. pictorum*, *S. rhizophila*, *S. terrae*, *S. maltophilia*, and *S. tumulicola* (https://lpsn.dsmz.de/genus/stenotrophomonas) (Figure 1). Further, there are species namely *S. cyclobalanopsidis*, *S. nematodicola*, *S. panacihumi*, and *S. sepilia*, which are yet to be validated (https://lpsn.dsmz.de/genus/stenotrophomonas).

Figure 1: 16s rRNA gene sequence-based phylogenetic tree of 20 valid species of *Stenotrophomonas* genus.

History

Stenotrophomonas maltophilia was first isolated in 1943 by J. L. Edwards as *Bacterium bookeri* in England from the pleural effusion of a patient [11]. He mentioned that it might be a skin contaminant and a non-motile bacterium. In 1953, Hugh characterized six strains of bacteria and found the presence of multitrichous flagella [11]. Later it was classified as *Pseudomonas maltophilia* [12]. In parallel, *Pseudomonas melanogena* isolated from Japanese rice paddies in 1963 was also later documented as *Pseudomonas maltophilia*. Furthermore, rRNA cistron analysis reclassified it as a member of the genera *Pseudomonas* and *Xanthomonas* in 1961 and 1983, respectively until it was classified as a new genus, *Stenotrophomonas*, in 1993 [10]. In a large study on *Xanthomonas* strains, an analysis of 295 phenotypic characteristics resulted in 7 strains being identified as *X. maltophilia*, with 2 of these 7 being type strains of *Pseudomonas betle* and *Pseudomonas hibiscicola* [13]. The DNA-rRNA hybridization studies, sequencing and mapping of PCR-amplified 16s rRNA genes have proposed a genus *Stenotrophomonas* with only one type species *S. maltophilia* in 1993 [10].

Genome characterization

In the genomic era, our understanding of bacterial taxonomy is based on taxonogenomics and phylogenomics provides the resolution at species and strain levels. *Stenotrophomonas maltophilia* genomes display extreme diversity at the inter-strain level [14]. Over the years, *S. maltophilia* has shown how an opportunistic pathogen can evolve into a unique sequence type that poses a significant concern in clinical settings. Genome dynamics such as recombination and selection pressure are two crucial traits in the evolution of microorganisms required for adapting to new hosts, immune systems, and antimicrobial resistance. The genomics database has more than 1000 whole genome sequences of *S. maltophilia*. The complete genome sequence of environmental isolate *S. maltophilia* R5513, and a clinical isolate *S. maltophilia* K279a, has shown genomic diversity in adaptation to different niches [15]. *Stenotrophomonas maltophilia* K279a strain is a multidrug-resistant clinical isolate 4,851,126 bp in length, with an average G+C content of 66.32%, and no plasmid. Comparative genomic analysis of the clinical and endophytic *S. maltophilia* isolates K279a and R551 showed a similar level of antibiotic resistance and utilisation of trehalose sugar. Moreover, both strains had genes encoding for type I and type VI pili for adhesion/adherence/auto-aggregation, and twitching motility, respectively. The K279a has codes for five hemagglutinins open reading frame fragments of type VI pilus, which are specific and might be associated with niche adaptation or host preference [15].

According to Gröschel and co-workers (2020), the *S. maltophilia* complex can be divided into 23 lineages, two comprising exclusively environmental strains [16]. The remaining lineages contain strains from mixed environmental and human sources. Lineage Sm6 comprises the highest number of human-associated strains, linked to key virulence and resistance genes. These *S. maltophilia* complex lineages are further divided into four more distantly related lineages (Sgn1-4) and several *S. maltophilia sensu lato* and *sensu stricto* lineages. The *S. maltophilia* strain K279a, isolated from a patient with bloodstream infection, serves as an indicator strain of the lineage *S. maltophilia sensu stricto* [16]. Phylogenetic analyses of 375 non-duplicated *S. maltophilia* complex genomes (including 226 of human, 30 of environmental, 104 of animal origin, and 15 of unknown origin) identified at least 20 genogroups; MLST analysis has shown that most strains in genogroups 1, 3, 6, and C, are of human origin, and most of the strains in genogroups 2-b and 5 are of animal origin [1].

Virulence traits

Stenotrophomonas maltophilia was recognized as low pathogenic bacteria. As science evolved, many investigations revealed the previously unknown *S. maltophilia* virulence components. Although this pathogen has a low level of virulence, it is well-

equipped with various virulence factors, including extracellular enzymes, and protein secretion systems that facilitate protection against host defenses. Secondary structures like pili and fimbriae are responsible for motility and attachment, respectively. The ability of *S. maltophilia* to produce biofilm and its innate antibiotic resistance has garnered much attention in recent years, making it therapeutically challenging (Figure 2). Protein secretion systems (PSS) are the structures present in the cell membrane of bacteria to release the virulence factors to affect the host. *Stenotrophomonas maltophilia* harbor Xps type II PSS to secrete various proteins including extracellular enzymes and virulence factors into its external environment**.** Clinical isolates of *S. maltophilia* have varying degrees of Xps type II PSS expression, suggesting that this system may facilitate *S. maltophilia* dissemination *in vivo* and cause host lung injury [17]. Another PSS in the *S. maltophilia* is VirB/D4 type IV PSS which is conserved among various strains and mediates the killing of human and other bacterial cells. Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella enterica* serovar Typhi, have recently succumbed to *S. maltophilia* K279a in a type IV secretion system-dependent manner. Additionally, other species can recognise secreted effectors from one species and transport them using the other species' type IV secretion system [18].

Figure 2: Antigenic structure of *Stenotrophomonas* bacteria.

Cell surface structures (flagella, pili, non-pilus adhesions etc.) are responsible for motility, adherence and colonization of a pathogen on the host surface and abiotic surfaces in clinical settings, thus acting as important virulence factors. *Stenotrophomonas maltophilia* carries single or multiple polar flagella that facilitate the motility and attachment inside the host. The flagellin protein of *S. maltophilia* confers early-stage adhesion, and the anti-flagellin serum could prevent adhesion in a dose-dependent manner.

Pili are the small fibrillar structures that facilitate bacteria to attach directly to their target eukaryotic cells. Additionally, they function as "bridges" between bacterial cells, which helps in colonization, and influences pathogenicity and horizontal transfer of antimicrobial-resistant genes. It has been proven that fimbriae deficient mutants are less virulent than the parent strains, which is likely because bacteria and epithelial cells are unable to communicate effectively.

Biofilm formation

Biofilm production enables bacteria to survive and persist on various abiotic surfaces of the hospital environment. It is an important characteristic of *S. maltophilia* and provides it with greater resistance against a wide range of antimicrobials. However, the expression of resistance depends upon the type of antimicrobial agent, the substrate's composition, and the biofilm's age [19]. There are reversible and irreversible stages of bacterial aggregation and biofilm formation, each incorporating a variety of unique variables. The development of biofilm involves two fundamental steps. Bacteria bind to the surface in the first phase, followed by bacterial aggregation and the development of multilayer structures in the second phase. It comprises a densely packed bacterial population and its extracellular matrix that contains amyloidogenic proteins, exopolysaccharides, and extracellular DNA. Di Bonaventura and co-workers (2007) showed that *S. maltophilia* SM33 cells could adhere within 2 hours to polystyrene surfaces and form biofilms within 24 hours of inoculation [20]. It can form biofilms on wet surfaces like dental suction tubing, catheters, respiratory tubing, clinical sink drains, dialysis, and water plumbing systems. The horizontal gene transfer phenomenon is more in biofilms than in planktonic cells. Sodium phosphate can be used to inhibit the biofilms of clinical *S. maltophilia* isolates [21].

Temperature, pH, CO_2, glucose concentration, iron limitation, and static or dynamic circumstances all have an impact on the production of biofilms in *S. maltophilia* [19]. Biofilm formation by clinical *S. maltophilia* isolates has been seen at 32°C to be more pronounced than at 18°C and 37°C [20]. In comparison to anaerobic settings, the production of biofilm was greater under aerobic conditions and in an environment with 6% CO_2. Comparable biofilms were generated by the *S. maltophilia* isolates at *p*H 8.5 and 7.5, while a thicker biofilm developed at *p*H 5.5. [20].

Poly-microbial biofilm phenomenon often found in gram-negative lactose non-fermentative (NLNF) group of pathogens that includes *S. maltophilia*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and sometimes *Burkholderia cepacia*. This interspecies interaction is a complex yet important phenomenon to enable infection in the host body [22]. Numerous studies on *P. aeruginosa* and *S. maltophilia* co-isolation suggest that the mystery of their coexistence is still being unravelled.

Antimicrobial resistance

Stenotrophomonas maltophilia harbours various mechanisms for antimicrobial resistance such as over-expression of multidrug efflux pumps, integrons, insertion sequence common region (ISCR) elements, antibiotics modification, SmQnr determinants, and plasmid-mediated resistance [2,4,6]. These mechanisms have provided an intrinsic resistance of *Stenotrophomonas* to a wide range of antibiotics including fluoroquinolones, carbapenems, β-lactams, aminoglycosides, trimethoprim-sulfamethoxazole (TMP-SMX), chloramphenicol, macrolides, tetracyclines and polymyxins [2,6].

Traditionally, TMP-SMX was regarded as the first line of treatment for *S. maltophilia* infection [4]. However, the susceptibility pattern has changed over some time. A large-scale study on 1,586 isolates of *S. maltophilia* from globally diverse medical centers revealed a decreasing susceptibility pattern for TMP-SMX throughout the world, which ranges from 1.1% in Europe, 2.4% in North America, 4.5% in Latin America to 9.2% in Asian-Pacific regions [23]. Similarly, the results of the SENTRY antimicrobial surveillance program of pneumonia patients, in the United States (U.S.) and European hospitals, from 2009 to 2012 demonstrated that 96.3% of 302 *S. maltophilia* isolates were sensitive to TMP-SMX [24]. Since then, the 130 isolates recovered from a U.S. center displayed a reduction in the susceptibility pattern ranging from 79% to 96% in 2016 and 2006, respectively [25]. Alteration of the intrinsic resistance in *S. maltophilia* affects the mutant selection window and suggested that inhibition of this intrinsic resistance could result in the selection of *S. maltophilia* antibiotic-resistant mutants at low antibiotic concentrations.

Fluoroquinolones are broad-spectrum antibiotics and, have been used as an alternative therapeutic option against multidrug resistance (MDR) *S. maltophilia* infections despite having adverse side effects. In fluoroquinolones, levofloxacin has shown a high clinical efficiency thus frequently used in clinical cases [26]. However, recently, reports of levofloxacin resistance have emerged. Wu and co-workers (2022) recently, revealed that TMP/SMX-resistant *S. maltophilia* isolates often exhibited concurrently with levofloxacin resistance and *vice-versa* [27]. Quinolone resistance in *S. maltophilia* is associated with the overexpression of intrinsic multidrug-resistant efflux pumps SmeDEF and SmeVWX, and plasmid-mediated qnr genes. These efflux systems also provide cross-resistance against tigecycline, chloramphenicol and tetracycline [28].

A retrospective cohort study was done by collecting microbiological data in a tertiary-care university hospital in Hungry revealed that out of 579 S *maltophilia* isolates collected between January 2008 and December 2017, 12.1% were SMX/TMP-resistant (2008-2012: 6.12%, 2013-2017: 18.06%; p = .034), while 8.99% were resistant to levofloxacin (2008-2012: 7.86%, 2013-2017: 10.12%; p > .05) [29]. Similar observations were recorded in the cross-sectional study of 12-month duration from 2017 to 2018, out of 117 *S. maltophilia* isolates, 10.25% were resistant to TMP/SMX, 16.1% were resistant to minocycline, and 27.11% were resistant to chloramphenicol and ceftazidime [30].

Efflux pumps

The resistance nodulation division (RND) family, ATP-binding cassette (ABC) family, and major facilitator superfamily (MFS) are the important MDR efflux pump groups that have been studied in *S. maltophilia*. The RND family, however, is the most common efflux pump family that is present in *S. maltophilia* including members like SmeABC, SmeDEF, SmeGH, SmeIJK, SmeMN, SmeOP, SmeVWX, and SmeYZ (Table 1). Some of these efflux systems are suggested to play an important role in the intrinsic resistance of *S. maltophilia* because, under normal growth conditions, they have a basal expression level sufficient to lower bacterial susceptibility to antibiotics. Additionally, the efficacy of antibiotics against *S. maltophilia* is increased by their inactivation.

Identification of *S. maltophilia*

Laboratory diagnosis of *S. maltophilia* in routine microbiological practice is difficult and often limited to NFGNB. Emergence in clinical cases accelerated the advancement of laboratory isolation

S. No.	Efflux pump	Antibiotic resistance
1	SmeDEF (SmeT negative regulator)	Quinolones, chloramphenicol, tetracycline, macrolides, sulfamethoxazole, trimethoprim and co-trimoxazole
2.	SmeGH (Intrinsic)	Beta-lactams, quinolones, tetracycline, and polymyxin B, toxic agents (menadione, tert-butyl hydroperoxide, naringenin and hexachlorophene)
3.	SmeIJK (Intrinsic)	Aminoglycosides, tetracycline, ciprofloxacin, levofloxacin, and minocycline
4.	SmeYZ (Intrinsic)- SmeSyRy regulator	Aminoglycosides and co-trimoxazole.
5.	SmeABC	Aminoglycosides, beta-lactams, fluoroquinolones, ciprofloxacin
6.	SmeVWX pump	Quinolones, chloramphenicol, tetracyclines, co-trimoxazole

Table 1: Major efflux pumps responsible for resistance in *S. maltophilia.*

and identification of *S. maltophilia*. Combinations of various antibiotics and antifungal agents have been used for selective isolation. Initially, *Xanthomonas maltophilia* selective medium (XMSM) was developed for the isolation of *S. maltophilia* from soil and rhizosphere environments. That contains a selective combination of six antibiotics (bacitracin, cephalexin, neomycin, novobiocin, penicillin G, and tobramycin), two antifungal agents (cycloheximide and nystatin), maltose, and bromothymol blue indicator [31]. Acid production might be seen as yellow colour halo formation around the *S. maltophilia* colony however, the colour may fade with extended incubation. To overcome this, Kerr and co-workers (1996) developed a VIA medium (vancomycin, imipenem, and amphotericin B) consisting of mannitol base and bromothymol blue indicator for the selective cultivation of *S. maltophilia* from clinical and environmental samples [32]. *Stenotrophomonas maltophilia* does not ferment mannitol, therefore, produces green coloured colony with a blue halo. It was proven to be superior to *Xanthomonas maltophilia* selective medium (XMSM) in terms of simplicity, affordability, specificity, and sensitivity [31]. Later in 2007, Foster (2008) modified the VIA medium by replacing imipenem with meropenem at a half concentration (16 mg/L) [33]. Even though this modification couldn't match the original VIA medium's specificity, the modified VIA provides an acceptable substitute for XMSM and VIA, when imipenem is not available. The VIA medium showed 80-100% of selectivity, and frequently being used for selective isolation of the *S. maltophilia* from sputa and soil samples. Approximately 80% of *S. maltophilia* formed smooth, round, green-coloured colonies surrounded by a blue-coloured halo on VIA medium [34]. The VIA medium is selective but sometimes, a resistant strain of other NFGN bacteria can grow on the medium that can be further ruled out by various biochemical tests. *Stenotrophomonas maltophilia* forms pale-coloured small non-lactose fermenting colonies on the differential media like MacConkey lactose media. Lavender green-coloured non-hemolytic small colonies on blood agar. It is oxidase negative, positive for catalase, esculin hydrolysis, tween 80 hydrolysis, produces H_2S in protein medium, and maltose fermentation, and produces gelatinase and DNAase, lysine decarboxylases, and motility in semisolid media. This Gram-negative bacillus is 0.7-1.8 × 0.4-0.7 µm in size and covered with a febrile structure of 5 to 7 nm. Two or more polar flagella of 40 to 50 nm in size are also present to provide motility [10]. Species-specific (SS) identification of *S. maltophilia* relies on 16rRNA gene sequencing, polymerase chain reaction (PCR), real-time PCR, and next-generation sequencing (NGS) techniques. 16S rRNA gene is the marker of reference in bacterial classification and was used to classify NFGN bacteria [2].

It has been challenging to distinguish *S. maltophilia* due to its high genetic diversity; few pieces of research have been conducted to identify a precise gene target for the diagnosis. Clinical strains of *S. maltophilia* isolated from CF sputum, identified by species-specific polymerase chain reaction (SS-PCR) based on the 23s rRNA gene demonstrated 100% specificity. But in environmental samples, the same primers have not demonstrated repeatability [34]. The *sme*DEF operon was the first multidrug efflux pump reported in *S. maltophilia* that contributes to intrinsic resistance

to quinolones, tetracycline, macrolides, chloramphenicol, and novobiocin was studied by using the *sme*D gene. Later, it was discovered that *sme*D gene is an SS marker for *S. maltophilia* and was employed in the development of multiplex PCR to distinguish between *S. maltophilia* and *S. rhizophila* [35]. According to the evaluation by Pinot and co-workers (2011), *smeD* could amplify only in *S. maltophilia* and not in *S. terrae, S. nitritireducens*, *S. humi* and *S. acidaminiphila* [34]. Furthermore, Okuno and colleagues (2018) targeted another maker i.e., *sme*T gene as an alternative identification marker of *S. maltophilia*. The *sme*T gene is a part of SmeDEF pump and is responsible for its regulation [36]. The *sme*T gene primers have been tested only on 22 *S. maltophilia* isolates from cheese and other milk products; therefore, more evaluation studies are required to determine the efficiency of this gene. To date *sme*D and 23s rRNA gene primers are promising candidates for early screening of *S maltophilia*; moreover, various molecular typing methods have evolved over the decades for early investigation of the pathogen in epidemiological outbreaks. The rapid detection of *S. maltophilia* in respiratory samples from pneumonia patients using a loop-mediated isothermal amplification (LAMP) assay showed no cross-reactivity with other tested bacteria and, most notably, no interference with amplification when the bacteria were mixed with organic compounds [37].

Diagnostic DNA microarrays and peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) allowed the detection of *S. maltophilia* in intracranial bacterial/fungal infections and tracheal aspirate and bronchoalveolar lavage samples [38]. Application of the PNA FISH assay may be useful for studying the pathogen in its biofilm within chronically colonized patients. Metagenomics sequencing has been used to identify *S. maltophilia* in spinal cord aspirate and blood samples from pediatric patients, enabling subsequent successful antimicrobial therapy and positive outcomes [2].

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has also been used to identify patient isolates of *S. maltophilia* [39] and to identify bacterial pathogens in groundwater. For the identification of clinical *S. maltophilia*, MALDI-TOF MS has demonstrated a high concordance rate with 16S rRNA gene sequencing and superiority over conventional phenotypic identification.

Molecular typing methods

For many years, typing of bacterial isolates has been utilized in national and international surveillance programs to track newly emerging resistant clones as well as to analyze local epidemics. The repetitive-sequence-PCR (rep-PCR), pulsed-field gel electrophoresis, multilocus variable number tandem repeat analysis, and multilocus sequence typing [25] are the most popular typing techniques used for clinical epidemiology of *S. maltophilia* strains.

Despite the advancement in phenotyping and genotyping techniques, PFGE is still the acknowledged gold standard for DNA fingerprinting [40]. The PFGE typing of the 154 isolates of *S. maltophilia*, demonstrated that 22 strains of eight different pulse types and 132 isolates had unique patterns [40]. Jumaa and co-workers (2006) used PFGE typing for *S. maltophilia* isolated from bacteremia cases that occurred during 2000-2004, in a tertiary referral hospital, in the United Arab Emirates. They found that duplicate isolates of *S. maltophilia* had indistinguishable PFGE profiles, supporting the validity and reproducibility of PFGE as a fingerprinting method [41]. Since PFGE depends upon sophisticated equipment and requires highly skilled personnel and time, it is not suitable for all clinical laboratories. Thus PCR-based fingerprinting methods provide a valid alternative for PFGE with the same discriminatory power successfully used to distinguish *S. maltophilia* strains.

Restricted fragment length polymorphisms (RFLP), targeting the *gyr*B gene, grouped about 191 *S. maltophilia* isolates into nine different clusters and showed distinct and unique patterns [42]. The *gyr*B gene is an essential part of DNA replication and is present in all bacteria as a single copy, have been used to estimate the phylogenic relationship between various bacteria [2]. Amplified fragment length polymorphism (AFLP) fingerprinting by using DNA hybridization and 16s rDNA sequencing could subdivide *S. maltophilia* into ten AFLP genomic groups including 100 clinical and environmental isolates [43]. The recent most relevant AFLP method is repetitive sequence PCR, which is further divided into three types, enterobacterial repetitive intergenic consensus (ERIC) PCR, BOX, and repetitive extragenic palindromic (REP) PCR. These are the small repetitive DNA sequences present throughout the genome of bacterial genes and utilized to compare the diversity of the isolates from different sources.

The rep-PCR primers are complementary to repetitive sequences. Using PCR, this method amplifies diverse regions of DNA flanked by the rep sequences, leading to amplicon patterns specific to subgroups within the type. The ERIC-PCR patterns with differences of two or more DNA bands are considered different strains, whereas isolates with differences of just one band indicate subtypes. Isolates are regarded to have the same pattern by computer-assisted analysis of ERIC-PCR patterns if the similarity coefficient between their patterns equals or exceeds 93% [44]. Lin and co-workers (2008) compared the dendrograms of 20 *S. maltophilia* strains prepared from PFGE and rep PCR (ERIC, BOX, and REP). He found that while BOX-PCR and REP-PCR revealed equivalent discriminatory power for closely related, genetically linked isolates, ERIC-PCR showed a much-reduced discriminatory power compared to PFGE [45].

Epidemiology

Animal cases

Stenotrophomonas maltophilia is emerging as an opportunistic veterinary pathogen. This neglected pathogen has received a lot of attention due to its antimicrobial resistance potential and, difficulty in treatment. This bacterium can cause a variety of clinical problems in immunocompromised animals, including skin and soft tissue infections, respiratory tract infections, severe septicemia, and cardiovascular infections (Table 2).

S. No.	Cases	Host Species	Place	Reference
1.	Lymphadenitis	Omani Goat	Oman	[46]
2.	Milk (mastitis)	Cattle	Japan	[2]
3.	Tracheobronchial wash	Equine	Greece	[47]
4.	Pyogranulomatous hepatitis	Buffalo	Greece	[48]
5.	Septicemia	African dwarf crocodile	Nebraska – Lincoln	[49]
6.	Fleece rot	Sheep	Australia	[50]
7.	Healthy milk and products		Egypt	[51]
8.	Subacute ruminal acidosis	Cattle	China	[52]
9.	Lower urinary tract infection	Canine	Czech Republic	[53]
10.	Skin Sarcoptic ulcer	Swine	Kerala, India	[54]
11.	Focal mucopurulent placentitis	Equine	USA	[55]
12.	canine prostate gland Infertility	Canine	Poland	[2]
13.	Co-infection with Influenza	Swine	Brazil	[2]
14.	Subclinical mastitis	Cattle	India (Gujarat)	[2]
16.	Cutaneous, respiratory & urine	Equine, Canine, Cat, Reptile	France	[2]
18.	Mucopurulent exudate in the trachea	Equine	Denmark	[2]
19.	Healthy, faecal microbiome	Vulture (California condor)	USA	[2]
20.	Fin rot	African catfish *Clarias gariepinus*	West Bengal	[2]
21.	Gill	Healthy rainbow trout	Iran	[2]
22.	Conjunctival flora	Turtles	Italy	[2]
23.	Cloaca	Prairie rattlesnake	USA	[2]

Table 2: *Stenotrophomonas maltophilia* reports in animals.

[updated from https://doi.org/10.1128/CMR.00030-19]

Stenotrophomonas maltophilia is often associated with respiratory disorders in animals. In a study [47], strains of *S. maltophilia* were isolated from the airways of horses, dogs, cats, and pythons, suffering from chronic respiratory disorders. A total of five strains were derived from them, with isolates 1, 2, and 3 from horses, isolates 4 from a dog, and isolate 5 from a cat. In Denmark, there was a clinical history of persistent coughing, and endoscopy revealed a significant amount of mucopurulent exudate in the lower trachea of three horses. Grey, slow-growing colonies

on tracheal aspirate culture were identified as *S. maltophilia* by 16s ribosomal DNA sequencing and API 20NE identification. It can also cause co-infection with other respiratory viral infections like the influenza virus in pigs [2].

Stenotrophomonas maltophilia also has the potential to cause infection associated with the reproductive system and thus able to influence fertility in animals. In Poland, a 4-year-old male Jack Russell Terrier with a history of conception failure was found positive for *S. maltophilia* in prostatic fluid. After the antibiotic treatment with ciprofloxacin, the animal recovered from infertility. Furthermore, two cases of equine focal mucopurulent placentitis were associated with *S. maltophilia*. The bacteria were isolated from the exudate of both mares and the lungs and stomach of the fetus [2].

A case series of three recurrent urinary tract infections (UTI) in dogs was reported in the Czech Republic. Histological examination of biopsy specimens collected during cystoscopy revealed chronic urocystitis. The culture of the specimen revealed *S. maltophilia* infection [53]. A Lymphadenitis outbreak was reported in Omani goats, associated with *S. maltophilia* infection, in 16 goats showing external abscesses ranging in size from 2-10 cm in diameter. This outbreak occurred in the goats maintained in a closed housing with 15-20 animals together [46].

The 1st case of *S. maltophilia* infection in buffalo was reported from Greece; the 7-year-old female buffalo was suffering from pyogranulomatous hepatitis. The author stated that the buffalo was not immunosuppressed thus this case suggested an independent mono-infection of *S. maltophilia* in healthy animals [48]. *Stenotrophomonas maltophilia* was isolated in pure culture from the kidney, lung, and liver of a 17-year-old male crocodile (captive African dwarf breed). The crocodile died due to septicaemia due to *S. maltophilia*, vegetative valvular lesions on the left atrioventricular valve and necrotic lesions on the myocardium [49].

In Japan, a bovine mastitis outbreak of a genetically related strain of *S. maltophilia* was reported. In India, *S. maltophilia* was isolated from pigs' skin ulcers that were associated with sarcoptic mange in Kerala. Five pigs with skin lesions were subjected to swabs collection and processing. The isolated *S. maltophilia* organisms shared similar cultural appearances and biochemical traits [54]. A subclinical mastitis case was reported in a cow from Gujarat. Recently (ICAR-Indian Veterinary Research Institute, Annual Report, 2022), *S. matophila* has been reported as a cause of septicaemia followed by death in a zoo leopard in Northern India. Apart from mammals, *S. maltophilia is* often isolated as commensal from the lower marine vertebrates, insects, protozoa, nematodes and reptiles. it was found to cause fin rot in the African catfish *Clarias gariepinus*. Lower invertebrates harbouring *S. maltophilia* can act as a vector for transmission to mammals. Faulde and Spiesberger (2013) suggested moth fly carrying *S. maltophilia* could be a mechanical vector in a German hospital [2]. However, a systematic study is required to determine the role of animals in transferring disease to humans.

Humans

Several infectious illnesses have been documented to have *S. maltophilia* as an etiological agent in humans. Most of the human cases associated with *S. maltophilia* infection are respiratory infections and bacteremia, furthermore, this bacterium is very versatile and has been reported to cause various infections in human systems like ocular infection, liver infection, medical implant infection, gastrointestinal tract infections, nervous system and spinal cord infections and urinary tract infections [2]. Biofilm-forming ability on medical instruments and associated environment, made them a common cause of outbreaks in ICUs, with a reported frequency of 7-38 cases/10,000 discharges in nosocomial settings. Studies have also highlighted the importance of these bacteria in community-acquired illnesses [29]. *Stenotrophomonas maltophilia* often infected the patient with COVID-19 during the pandemic as a secondary bacterial infection [9]. In our view contrary to animals, human reproductive system infections have not been documented [2,56]. It is one of the organisms causing secondary infections in cystic fibrosis patients [3]. The crude mortality rate for invasive *S. maltophilia* infections is quite high, especially if the patients receive inappropriate empiric therapy: 20-60% in the case of bacteremia/sepsis and 20-70% in the case of pneumonia [57]. Common risk factors that are associated with the mortality rate in a patient infected with *S. maltophilia*, are ventilation [58], septic shock and length of stay in the hospital [59].

Environment

Stenotrophomonas maltophilia is a versatile pathogen and is ubiquitously found in various natural and anthropogenic

environmental sources [15]. It is found in the rhizosphere of plants and produces phytohormones with antagonistic capabilities against bacterial and fungal plant diseases and activity that promotes plant growth, and chitinolytic activities. It has been widely recovered from various water sources, soil [56] and sewage water (IVRI, Annual Report, 2022). This pathogen is known for its inherent ability to degrade various hydrocarbons, and xenobiotics and thus has been used in bioremediation [2].

Stenotrophomonas maltophilia - a food-borne pathogen

Stenotrophomonas maltophilia has been associated with plant and animal-based food products. Salads and green vegetables are widely consumed raw and *S. maltophilia* has been recovered from ready-to-eat salads and vegetables. It was also found in samples of ready-to-eat food taken from three different street food markets in Taiwan. The presence of *S. maltophilia* in human consumable milk and milk products has also been reported *Stenotrophomonas maltophilia* has been reported as a contaminant in raw milk samples, cheese and ice cream samples. Water could be the major source of *S. maltophilia* contamination in food products and edible ice samples. It is isolated in the sanitized milk processing unit as a contaminant. *Stenotrophomonas maltophilia* showed the highest survival rate in alcoholic beverages like vodka [2]. Recently, it was isolated from poultry meat in Japan [60], and Neem (*Azadirachta indica*) leaves eaten raw [61], Food products carrying *S. maltophilia* are also a source for multidrug resistance gene transmission thus continuous monitoring is required to implicate the preventive measures.

Conclusion

Stenotrophomonas maltophilia is an organism of concern due to its very dynamic characteristics. They are unique with both colonization and true pathogen in immunocompromised individuals. Owing to its presence in various paraclinical environments, debilitating patients are much more vulnerable to these bacteria. Intrinsic antimicrobial resistance of the pathogen is an important concern not only for the difficulty in cure of its infection but also for the transfer of the AMR genes to other bacteria. Researchers are focusing on the possibilities of distinguishing the beneficial and harmful *Stenotrophomonas* strains into different species. This could widen the gates of biotechnological applications of this versatile bacterium and for the development of appropriate treatment strategies. A complete understanding of epidemiology, drug resistance, alternative treatment strategies and adaptation to various environments are fascinating challenges for the future.

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Conflict of Interest

No conflicts.

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