Journal of International Case Reports (ICARE)
Volume 1 | Issue 3 | Pages: 94-99
Published Date: December 20, 2022

First Report of Microbiological and Molecular Detection of *Stenotrophomonas maltophilia* from Free-Range Leopard (*Panthera pardus pardus*)

Himani Agri, Ravichandran Karthikeyan, Varsha Jaykumar, Akanksha Yadav, Bhimavarapu Kiranmayee, Mathesh Karikalan, Abhijit M Pawde and Bhoj R Singh*

Division of Epidemiology, ICAR-Indian Veterinary Research Institute, India

Received Date: November 07, 2022; Accepted Date: December 04, 2022; Published Date: December 20, 2022

***Corresponding author:** Bhoj Raj Singh, Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar, 243 122, India, E-mail: brs1762@gmail.com

Citation: Himani Agri, Ravichandran Karthikeyan, Varsha Jaykumar, Akanksha Yadav, Bhimavarapu Kiranmayee, Mathesh Karikalan, Abhijit M Pawde and Bhoj R Singh. First Report of Microbiological and Molecular Detection of *Stenotrophomonas maltophilia* from Free-Range Leopard (Panthera pardus pardus). ICARE. 2022;1(3):1018.

Abstract

Stenotrophomonas maltophilia is a globally emerging pathogen. We report here the first case of *Stenotrophomonas maltophilia* associated septicaemia and death of leopard (*Panthera pardus pardus*). The bacterium was isolated in pure culture from lung tissue of the leopard with thoracic wounds. The isolate belonged to the same clade, confirmed through 16s rRNA gene sequencing, of *S. maltophilia* that was reported to cause mastitis in a cow in Gujarat in India and had similar antimicrobial drug resistance pattern indicating the potential of *S. maltophilia* to infect several hosts.

Keywords: Emerging pathogen; MDR; 16s rRNA gene sequencing; Phylogeny

Introduction

Stenotrophomonas maltophilia (Sm), an emerging pathogen [1], is a non-fermentative gram-negative bacillus that has previously been named as *Pseudomonas maltophilia* and *Xanthomonas maltophilia*. Primarily it is an opportunistic pathogen in humans mainly affecting immunocompromised patients. World Health Organisation (WHO) recognized this bacterium as one of the underestimated nosocomial pathogens and it was ranked as the ninth most important challenging pathogen in infectious disease studies. Over the past two decades, it transformed from non-pathogenic environmental colonizer to serious life-threatening organism. The natural reservoirs of *S. maltophilia* include the rhizosphere, freshwater and soil [1]. The major concern with *S. maltophilia* infections is its intrinsic resistance to multiple antibiotics and biofilm formation in various environments. Because of this pathogen's inherent resistance to various antibiotics, clinicians have few treatment options. This pathogen is either missed or misidentified in routine laboratory diagnosis due to its close resemblance with *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, *Acinetobacter baumanii* and *Alcaligenes faecalis*. With advancements in the identification of bacteria as Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS), Polymerase Chain Reaction (PCR), Real-Time PCR, Next-Generation Sequencing (NGS) techniques, Loop-Mediated Isothermal Amplification (LAMP), and Diagnostic DNA microarrays, the reports of *S. maltophilia* are on increase.

In animals the reports of *S. maltophilia* infections are limited and sporadic. It is reported both as primary and

secondary opportunistic pathogen of the respiratory tract [2,3] of horses, dogs, cats, and pythons, suffering from chronic respiratory disorders, [4] Reproductive and Urinary Tract Infections (UTI) in dogs [5,6] and also from milk and milk products [7]. In India, *S. maltophilia* is reported associated with subclinical mastitis in cows in Gujarat [8] and skin ulcers in pigs in Kerala. Here we report the isolation and identification of *S. maltophilia* from lung sample of a Leopard (*Panthera pardus pardus*) died of septicemic infection [9].

Materials and Methods

Bacterial Culture

The death of a male adult leopard with multiple injuries was reported at Moradabad Forest Department, Uttar Pradesh. The carcass was submitted to necropsy examination at the Centre for Wildlife Conservation, Surveillance and Management, ICAR - Indian Veterinary Research Institute, Izatnagar. Systemic necropsy examination revealed an open wound between 11^{th} and 12^{th} ribs with a fracture of the last thoracic vertebrae. Both the lungs' lobes had multiple consolidated patches with small pus pockets. All the visceral organs had severe vascular lesions like congestion and haemorrhages. The Lung Tissue (20 LG) was submitted to the Clinical Epidemiology Laboratory for the bacteriological examination.

The sample was initially plated onto 5% sheep blood agar, MacConkey agar (BBL-Difco, USA) and Vancomycin-Imipenem-Amphotericin B (VIA) agar [10] and incubated at 37°C for 24hrs to 48hrs. Well isolated 5 colonies were picked up for further characterization based on growth, staining and biological characteristics [11] for oxidase production, esculin hydrolysis, lysine and ornithine decarboxylaion, tween 80 hydrolysis, maltose fermentation, production of gelatinase, DNase, lecithinase, esterase, blackening of lead acetate paper on TSB (for H_2S), microscopic morphology and motility [12,13]. The isolate was processed for Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) based identification using the MALDI Biotyper® Sirius system.

Molecular Identification

The isolates were finally confirmed by sequencing 16S rRNA amplicons. To perform PCR, DNA extracted from freshly grown pure culture using the snap chill method was used as the template DNA, and primers used were 16s For-5'-GACTCCTACGGGAGGCAGCAG-3' and 16s Rev -5'-CTGATCCGCGATTACTAGCGATTC-3' targeting the 16s rRNA region. The amplification was performed for 35 cycles of denaturation, annealing, and extension at 94°C for one minute, 55°C for 30 seconds, and 72°C for two minutes respectively, with a final extension of 72°C for 5 minutes, PCR products were run on 1.2% agarose for gel electrophoresis and results were documented using Chemiluminescence gel documentation system (AlphaImager HP, Premas Life Sciences Pvt. Ltd New Delhi, India). The PCR product was custom sequenced from Eurofins Genomics India, New Delhi, India. The sequences were aligned using NCBI Basic Local Alignment Search Tool (BLAST) search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the matching sequences available in the GenBank database. For phylogenetic analysis, 16s rRNA gene sequences of different *S. maltophila* strains were retrieved from DNA Data Bank/ GenBank and aligned in MEGA 7.0.26 software. The best-fit model was identified by Model Finder [14] and a phylogenetic tree was constructed by the Maximum Likelihood method in the IQ-TREE 1.6.12 online platform [15,16]. The confirmed representative isolate was deposited in the repository of the Veterinary Type Culture Collection Centre (VTCC), ICAR-IVRI, Izatnagar for future reference.

All confirmed isolates were subjected to Antimicrobial Susceptibility Testing (AST) using the disc diffusion method on Mueller-Hinton agar (BBL-Difco, USA) as per CLSI guidelines [17]. The isolate was maintained on a nutrient agar slant till the end of the study.

Results and Discussion

The lung tissue received in the laboratory showed single type of colonies appeared on all three plating media after 24 hrs of incubation at 37°C. Colonies were lavender-coloured on 5% sheep blood agar, tiny transparent on MacConkey agar and green coloured with a blue halo on VIA agar (Figure 1). The visible β-haemolysis could be observed after

72 hrs of incubation at 30℃ on 5% sheep blood agar. Results of biochemical results (Table 1) were in concordance with those mentioned in Bergey's Manual of Determinative Bacteriology [18].

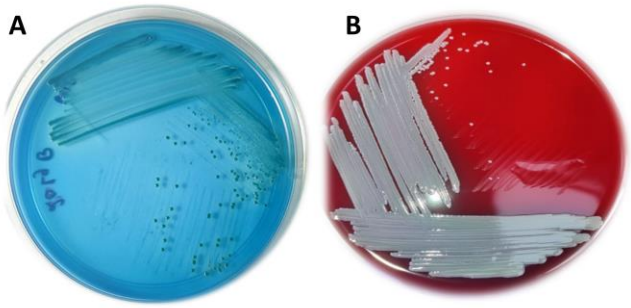

Figure 1: *Stenotrophomonas maltophilia* colony morphology. A: Green-coloured colony with blue-coloured halo on VIA agar medium at 30℃ for 24 hrs; B: Lavender green-coloured colony on sheep blood agar at 30℃ for 24 hrs.

Table 1: Biochemical characteristics of the *Stenotrophomonas maltophilia* isolates.

Biochemical tests	**Interpretations**	**Biochemical tests**	**Interpretations**
Esculin hydrolysis	Positive	Oxidase	Negative
Lysine and Ornithine decarboxylase	Positive	DNase	Positive
Maltose fermentation	Positive	Lecithinase	Positive
Tween 80 hydrolysis	Positive	Esterase	Positive
Gelatinase production	Positive	Blackening of lead acetate paper on TSB (for H2S)	Positive
Motility	Positive		

Stenotrophomonas maltophilia is reported both as a coloniser and disease-causing agent in animals. However, in animal clinical settings, it is difficult to distinguish contaminants from infections [19] but the isolation of *S. maltophilia* in pure culture from a leopard having an open wound in the thoracic cavity indicates the possibility of it being the main cause of infection without excluding chances as a secondary invader. The isolation of *S. maltophilia* from animals may not always be of contamination origin but may also be the cause of severe infections.

Antimicrobial Susceptibility Assay

All five isolates of *Stenotrophomonas maltophilia* tested in triplicate were resistant to most of the β-lactam antibiotics including imipenem. (Table 2) similar to those reported earlier [5-7]. The *S. maltophilia* bacterium carries a similar level of drug resistance in nature even while on herbaceous plants used as animal feed [20,21] and also when causing clinical infections [22]. The broad range of drug resistance often necessitates performing Antibiotic Susceptibility Tests (ABST) before initiation of the treatment especially in cases where chronic infections are present. The high levels of drug-resistance in *S. maltophilia* is thought to be due to presence of multiple efflux pump operons and resistance coding genes among both clinical and environmental isolates [23]. The isolates of *S. maltophilia* were susceptible to ceftazidime and ticarcillin-clavulanate, however, in ceftazidime susceptibility testing potential inaccuracies have been reported [22]. The susceptibility of the *S. maltophilia* isolates to Trimethoprim/Sulfamethoxazole (TMP/SMX), fluoroquinolones or a combination of β-lactam antibiotics β-lactamase inhibitors concurs with the recommendation of the use of these antimicrobials in animals [24,25]. However, resistance to TMP/SMX, a choice for veterinary therapeutic uses, is frequently reported in recent years [26-28].

Table 2: Antibiotic susceptibility assay results of the *Stenotrophomonas maltophilia* isolate.

Antimicrobials	**Results**	**Antimicrobials**	**Results**
Amikacin (30 µg)	S	Imipenem (10 µg)	R
Amoxycillin (30 µg) + clavulanic acid (10 µg)	R	Nitrofurantoin (300 µg)	S
Ampicillin (10 µg)	R	Tazobactam (10 µg)	R

Ceftriaxone (10 µg)	R	Tetracycline (30 µg)	S
Aztreonam (30 µg)	R	Kanamycin (30 µg)	S
Cefotaxime (10 µg)	R	Streptomycin (100 µg)	R
Colistin (10 µg)	S	Ertapenem(10 µg),	S
Polymixin B	S	Gentamicin (10 µg)	S
Ceftriaxone (30 µg)	R	Cefotaxime /Clavulanic acid (30 µg)	R
Ceftazidime (30 µg)	S	Ciprofloxacin (5 µg)	S
Erythromycin (15 µg)	R	Cefepime (30 µg)	R
Ticarcillin (75 µg)/clavulanic acid (10 µg)	S	Co-Trimoxazole 25 µg (23.75/1.25 µg)	S

Molecular Detection

The 16s rRNA sequence of the 934-bp amplicon, had 99.89% homology with *S. maltophilia* reference using the BLAST tool. The partial gene sequence of the amplicon was submitted to the NCBI (Accession number: OP199038). The phylogenetic analysis of the 16s rRNA partial sequence of *S. maltophilia* isolates using the maximum likelihood method created with TN+F+I model (Figure 2) identified the close relatedness of the isolate with other clinical and environmental isolates from India. The isolate in the present study belonged to the same clade as of *S. maltophilia* isolated from mastitis milk of a cow in Gujarat, India [8]. Further complete genome analysis of the isolate may provide novel findings. Recently, a comparative whole genome analysis of 104 *S. maltophilia* strains from animals with human and environmental strains supported the hypothesis that animals may be acting as a reservoir for human infections [29].

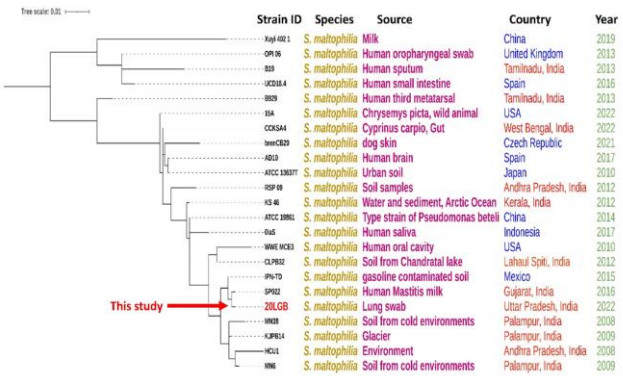

Figure 2: The phylogenetic tree based on partial sequence of 16S Ribosomal RNA gene with different *Stenotrophomonas maltophila* strains.

Conclusion

The study suggested that *S. maltophilia* might be circulating as a wide host range pathogen in India. *Stenotrophomonas maltophilia* and other non-fermentative gram-negative bacilli organisms should be considered as an important pathogen in routine clinical diagnosis, especially in the chronic form of any bacterial infections and affecting respiratory system.

Acknowledgement

Authors are thankful to the Director ICAR-Indian Veterinary Research Institute for permitting the study and funds used in the study. The first five authors also thank the Institute for providing scholarship and contingency funds during the study.

References

1. Agri H, Karthikeyan R, Kiranmayee B, Jayakumar V, Yadav A, Vinodh Kumar, et al. *Stenotrophomonas maltophilia:* An Overlooked Enemy Disguised as a Friend. Acta Scientific Microbiol. 2022;5(11):68-80.
2. Albini S, Abril C, Franchini M, Hussy D, Filioussis G. *Stenotrophomonas maltophilia* Isolated from the Airways of Animals with Chronic Respiratory Disease. Schweize Arch Tierheilkd. 2009;151(7):323-8.
3. Winther L, Andersen RM, Baptiste K.E, Aalbæk B, Guardabassi L. Association of *Stenotrophomonas maltophilia* Infection With Lower Airway Disease in the Horse: A Retrospective Case Series. Vet J. 2010;186(3):358-63.

4. Brooke JS. Advances in the Microbiology of *Stenotrophomonas maltophilia.* Clin Microbiol Rev. 2021;34(3):e00030-19.
5. Kralova-Kovarikova S, Husnik R, Honzak D, Kohout P, Fictum P. *Stenotrophomonas maltophilia* Urinary Tract Infections in Three Dogs: A Case Report. Vet Med. 2012;57(7):380-3.
6. Domosławska A, Zdunczyk S, Jurczak A, Janowski T. *Stenotrophomonas maltophilia* Isolated from Prostatic Fluid as an Infertility Factor in A Male Dog". Andrologia. 2017;49(10).
7. Zeinhom MM, Hassan GM, Salem HA, Corke H. Prevalence and Survival of *Stenotrophomonas* Species in Milk and Dairy Products in Egypt. Foodborne Pathog Dis. 2021;18(5):337-45.
8. Bhanderi BB, Jhala MK, Ahir VB, Bhatt VD, Joshi CG. Cultural and Metagenomic Based Identification of A Microbiome from Subclinical Mastitis in Cows. Veterinarski Arhiv. 2014;84(3):215-28.
9. Thenissery A, Chandran R, Ravindran R, Ravishankar C, Piriyath JH. Isolation and Identification of Emergent Multidrug-Resistant *Stenotrophomonas maltophilia* from Skin Ulcers of Sarcoptes-Infested Pigs. Vet Record Case Rep. 2022;10(2):e312.
10. Kerr KG, Denton M, Todd N, Corps CM, Kumari P, Hawkey PM. A New Selective Differential Medium for Isolation of Stenotrophomonas maltophilia. Eu J Clin Microbiol Infect Dis. 1996;15(7):607-10.
11. Carter GR. Diagnostic Procedures in Veterinary Microbiology, 2nd ed, Charles C; Thomas Publishers: Springfield; 1975.
12. Singh BR. Laptop for Microbiology Laboratory. Lambert Academic Publishing: Germany; 2009.
13. Kreig NR, Holt JG. Bergey's Manual of Systematic Bacteriology. Williams and Wilkins: Balitimore; 1984.
14. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. Nature Methods. 2017;14(6):587-9.
15. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum Likelihood Phylogenies. Mol Biol Evol. 2015;32(1):268-74.
16. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol Biol Evol. 2018;35(2):518-22.
17. Clinical and Laboratory Standards Institute. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, M45, 3rd ed. Clinical and Laboratory Standards Institute, Wayne, USA; 2015.
18. Palleroni NJ, Bradbury JF. *Stenotrophomonas*, A New Bacterial Genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983. Int J Syst Evol Microbiol. 1993;43(3):606-9.
19. Petridou E, Filioussis G, Karavanis E, Kritas SK. *Stenotrophomonas maltophilia* As A Causal Agent of Pyogranulomatous Hepatitis in A Buffalo (Bubalus bubalis). J Vet Diagnost Invest. 2010;22(5):772-4.
20. Furlan JPR, Pitondo-Silva A, Stehling EG. Detection of blaNDM-1 in *Stenotrophomonas maltophilia* Isolated from Brazilian Soil. Mem Inst Oswaldo Cruz. 2018;113(6):e170558.
21. Furlan JPR, Sanchez DG, Gallo IFL, Stehling EG. Characterization of Acquired Antimicrobial Resistance Genes in Environmental *Stenotrophomonas maltophilia* Isolates from Brazil. Microb Drug Resist. 2019;25:475-9.
22. Rhoads DD. *Stenotrophomonas maltophilia* Susceptibility Testing Challenges and Strategies. J Clin Microbiol. 2021;59(9):e01094-21.
23. Sarhan SR, Ibrahim OMS. In-vitro Study the Antibacterial Activity of Bacteriocin against *Stenotrophomonas maltophilia* and Evaluation its Synergism with Some Antibiotics. Adv Anim Vet Sci. 2018;6(12):556-68.
24. Mojica MF, Papp-Wallace KM, Taracila MA, Barnes MD, Rutter JD, Jacobs MR, et al. Avibactam Restores the Susceptibility of Clinical Isolates of *Stenotrophomonas maltophilia* to Aztreonam. Antimicrob Agents Chemother. 2017;61(10):e00777-17.

25. Ko JH, Kang CI, Cornejo-Juárez P, Yeh KM, Wang CH, Cho SY, et al. Fluoroquinolones versus Trimethoprim-Sulfamethoxazole for the Treatment of *Stenotrophomonas maltophilia* Infections: A Systematic Review and Meta-Analysis. Clin Microbiol Infect. 2019;25(5):546-54.
26. Sun E, Liang G, Wang L, Wei W, Lei M, Song S, et al. Antimicrobial Susceptibility of Hospital Acquired *Stenotrophomonas maltophilia* Isolate Biofilms. Braz J Infect Dis. 2016;20(4):365-73.
27. Bhavana MV, Joshi S, Adhikary R, Beena HB. Antibiotic Susceptibility Pattern of Burkholderia cepacia Complex and Stenotrophomonas maltophilia: A 5-year Analysis. Indian J Med Microbiol. 2017;35:318-9.
28. Elufisan TO, Rodriguez-Luna IC, Oyedara OO, Sanchez-Varela A, García VB, Oluyide BO, et al. Antimicrobial Susceptibility Pattern of Stenotrophomonas Species Isolated from Mexico. Afr Health Sci. 2020;20(1):168-81.
29. Mercier-Darty M, Royer G, Lamy B, Charron C, Lemenand O, Gomart C, et al. Comparative whole-genome phylogeny of animal, environmental, and human strains confirms the genogroup organization and diversity of the *Stenotrophomonas maltophilia* complex. Appl Environ Microbiol. 2020;86(10):e02919-19.