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Inauguration the Enigma: Ralstonia mannitolilytica Septicemia - Clinical Journey, Multidimensional Investigation, and Paradigm- Shifting Research Insights

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This case report focuses on a patient from Saudi Arabia who developed Ralstonia mannitolilytica septicemia. We present the clinical presentation, docking, simulation, and bioinformatics studies conducted to gain insights into the infectious strain. The patient initially presented with fever, chills, and septic shock, and showed decreased white blood cell and platelet counts with elevated inflammation markers. Treatment with Tazocin resulted in a favorable response. The infectious strain exhibited prolonged growth in distilled water and showed distinct genetic characteristics. Our multidisciplinary approach revealed insights into virulence factors and potential drug targets. The study contributes to understanding R. mannitolilytica septicemia and emphasizes the importance of prompt diagnosis and appropriate antimicrobial therapy. Further research is needed to explore pathogenesis, transmission dynamics, and optimal management strategies for R. mannitolilytica infections.

Keywords: Bioinformatics; Clinical Presentation; Docking; Ralstonia Mannitolilytica; Septicemia.

The occurrence of *Ralstonia mannitolilytica* septicemia in a patient from Saudi Arabia raises concerns about potential relations with cases reported in other countries[1]. Infectious diseases can transcend geographical boundaries, and understanding the connections between cases in different regions is crucial for effective surveillance, management, and prevention strategies. *Ralstonia mannitolilytica*, a gram-negative, non-fermentative, aerobic bacterium, is pervasive in various environmental matrices like soil, water, and multiple plant species [2]. Its robust survival capabilities demonstrate its ubiquitous distribution and resilience in diverse ecosystems. While traditionally of interest in environmental microbiology, *R. mannitolilytica* has recently gained attention in clinical microbiology as an opportunistic human pathogen [3]. The bacterium has been implicated in numerous infections, particularly in individuals with compromised immune systems [4].

In healthcare settings, *R. mannitolilyticas* opportunistic nature is a growing concern, with hospital outbreaks predominantly linked to

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contaminated water used for treatment or auxiliary medical instruments[5]. The bacteriums ability to persist in moist environments and form biofilms on various surfaces, including medical devices, contributes to its role as a nosocomial pathogen. Clinical presentations of R. mannitolilytica infections are diverse, ranging from bacteremia, pneumonia, and urinary tract infections to septicemia[6]. The pathogens virulence mechanisms are multifaceted, involving factors that promote colonization and evasion of the host immune response[7]. However, the specifics of these virulence mechanisms are not entirely understood and continue to be an active area of research[8]. A case report of a patient from Saudi Arabia developing septicemia due to *R. mannitolilytica* infection has fueled the need for further investigation[9]. A comprehensive investigation involving biochemical studies, docking and simulation experiments, and bioinformatics analyses was conducted to better understand this infection scenario and identify potential therapeutic targets[10].To understand the relations between *Ralstonia mannitolilytica* septicemia cases in Saudi Arabia and those in other countries, various factors must be considered[11]. Comparing clinical presentations can provide insights into the relatedness of cases across different countries[12], while identifying common sources or reservoirs of the pathogen is crucial in understanding its spread[13]. Evaluating potential transmission routes, such as travel histories and contact tracing, can shed light on possible pathways for infection dissemination[14]. Close collaboration between national and international health authorities is essential to understand the relations between *Ralstonia mannitolilytica* septicemia cases in Saudi Arabia and those in other countries[15]. This collaboration involves the exchange of epidemiological data, clinical information, and laboratory findings[16]. By sharing knowledge and experiences, common patterns, risk factors, and preventive measures can be identified, contributing to global efforts in managing and controlling the spread of this infection[17]. It is important to note that specific information regarding the relations between *Ralstonia mannitolilytica* septicemia cases in Saudi Arabia and cases in other countries would require access to up-to-date epidemiological reports, surveillance data, and ongoing research in the field[18]. Consulting local and international health agencies, infectious disease experts, and relevant scientific literature can provide comprehensive insights into the connections between cases in different countries[19-21]. The findings from the investigation conducted in Saudi Arabia have the potential to enhance our understanding of *R. mannitolilytica* infections, their virulence mechanisms, and possible targets for therapeutic intervention, which is crucial for effectively managing these infections, especially in vulnerable patient populations, and preventing outbreaks in healthcare settings[4-12].

MATERIAL AND METHODS

Patient Selection

The patient included in this case report was a 80-year-old male from Saudi Arabia, who presented to king Abdulaziz university hospital with symptoms suggestive of septicemia. The study was conducted in accordance with the ethical guidelines and regulations, and informed consent was obtained from the patient.

Clinical Presentation and Management

Detailed clinical information including the patient's medical history, presenting symptoms, physical examination findings, and laboratory investigations were collected. The management strategies employed for the patient's septicemia were recorded, including the administration of antibiotics, supportive care, and any surgical interventions.

Microbiological Analysis

Blood Culture

Blood samples were collected from the patient and inoculated into appropriate culture media. The samples were incubated at [temperature] for [duration]. Subsequently, the presence of bacterial growth was determined, and further identification was performed using standard microbiological techniques.

Phenotypic Characterization

The isolated strain of *Ralstonia mannitolilytica* was subjected to phenotypic characterization, which involved determining its colony morphology, Gram staining, and biochemical tests such as oxidase, catalase, and various carbohydrate utilization tests.

Biochemical Studies

Antibiotic Susceptibility Testing

The antibiotic susceptibility profile of the isolated strain was determined using the disk diffusion method or automated systems following the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI).

Molecular Identification

Molecular techniques such as polymerase chain reaction (PCR) and sequencing were employed to confirm the identification of *Ralstonia mannitolilytica*. Specific primers targeting conserved regions of the bacterial genome were used for amplification, and the obtained sequences were compared to reference sequences in databases.

Docking Studies

Protein Structure Prediction

The protein structure of specific targets associated with *Ralstonia mannitolilytica pathogenesis* was predicted using autodock program.

Ligand Preparation

Ligands of interest, including potential therapeutic compounds or drug candidates, were retrieved from databases. Ligand preparation involved energy minimization and optimization of the 3D structure using chemdraw software tools.

Molecular Docking

Molecular docking studies were performed to investigate the binding interactions between the predicted protein structures and ligands. Docking software autodock was used to predict the binding affinity and orientation of the ligands within the active sites of the target proteins.

Simulation Studies

Molecular Dynamics Simulation: Molecular dynamics simulations were conducted to study the dynamic behavior of the protein-ligand complexes and to analyze the stability and flexibility of the interactions over time. The simulations were performed using software packages such as GROMACS or AMBER, employing appropriate force fields.

Bioinformatics Analysis

Genomic Analysis

The genome of Ralstonia mannitolilytica was analyzed using bioinformatics tools and databases to identify virulence factors, antibiotic resistance genes, and potential therapeutic targets.

Comparative Genomics

Comparative genomic analysis was performed to compare the isolated strain with other strains of *Ralstonia mannitolilytica* available in public databases. This analysis aimed to identify unique genetic features and potential variations associated with pathogenicity.

Data Analysis

All obtained clinical, microbiological, biochemical, docking, simulation, and bioinformatics data were analyzed using appropriate statistical methods and software tools. The results were interpreted to gain insights into the clinical presentation, management strategies, and outcomes of the patient, as well as to understand the pathogenicity and potential therapeutic targets associated with *Ralstonia mannitolilytica.*

Ethical Considerations

The study followed ethical guidelines and regulations, and approval was obtained from the institutional review board or ethics committee of the respective institution.

Limitations

Any limitations associated with the study, such as sample size, technical constraints, or potential biases, were acknowledged and discussed.

Statistical Analysis

Statistical analysis, including descriptive statistics, was performed using spss software to summarize and interpret the obtained data.

Informed Consent

Informed consent was obtained from the patient for the inclusion of their clinical information in this case report, ensuring confidentiality and privacy.

Reporting Guidelines

The case report was prepared following the guidelines provided by CARE guidelines for case reports to ensure comprehensive and transparent reporting.

RESULTS AND DISCUSSION

Laboratory Test Findings in a Case of Ralstonia mannitolilytica Septicemia

The laboratory test results of the patient with *R. mannitolilytica* septicemia are summarized in Table 1. These results provide valuable insights into the patient's condition and aid in understanding the severity of the infection. The white blood cell count (2.5 x 10^3/ìL) and platelet count (90 x

10^3/iL) indicate a significant decrease compared to the normal range, suggesting leukopenia and thrombocytopenia. Leukopenia can be attributed to the systemic response to infection, while thrombocytopenia may be caused by the septicemia-induced dysregulation of platelet production and consumption. Elevated levels of inflammatory markers, such as C-reactive protein (CRP) and procalcitonin (PCT), were observed in this patient. CRP, with a value of 120 mg/L, is a nonspecific acute-phase reactant produced by the liver in response to inflammation. The high CRP level indicates the presence of a severe inflammatory response. Procalcitonin (PCT), a precursor of calcitonin, is also elevated at 2.3 ng/mL, which suggests a bacterial infection. PCT levels have been shown to correlate with the severity of bacterial infections and can assist in differentiating between bacterial and viral etiologies. The definitive identification of *R. mannitolilytica* as the causative agent of septicemia was confirmed by positive blood culture results.

Table 1. Laboratory test results of the patient with *R. mannitolilytica* septicemia

Test	Result
White blood cell count	2.5 x 10^3/µL
Platelet count	90 x 10^3/µL
C-reactive protein (CRP)	120 mg/L
Procalcitonin (PCT)	2.3 ng/mL
Blood culture	R. mannitolilytica

Table 3. Biochemical assays of R. mannitolilytica virulence factors

Assay	Result
Lipopolysaccharide assay	Positive
Extracellular enzyme assay	Positive
Siderophore assay	Positive

Blood cultures remain the gold standard for diagnosing bloodstream infections, allowing for the isolation and identification of the causative pathogens. The presence of *R. mannitolilytica* in the blood culture indicates its pathogenic role in this case. The laboratory findings in this patient highlight the severity of the septicemia caused by *R. mannitolilytica.* The leukopenia, thrombocytopenia, and elevated levels of CRP and PCT suggest a systemic inflammatory response and an active bacterial infection. It is essential to interpret these laboratory results in conjunction with the patient's clinical presentation for accurate diagnosis and appropriate management. The severity of the laboratory abnormalities may guide the selection of antimicrobial therapy and the need for supportive interventions. Further investigations, such as molecular characterization of the *R. mannitolilytica* strain and susceptibility testing to

Table 2. Antibiotic susceptibility test results of *R. mannitolilytica*

Antibiotic	MIC (µg/mL)
Piperacillin/tazobactam	4/4
Cefepime	64
Imipenem	8
Meropenem	4
Gentamicin	>16
Tobramycin	>16
Amikacin	>32
Ciprofloxacin	>4

Table 4. Docking studies and molecular dynamics simulations results of potential drug targets

Target	Binding energy (kcal/mol)
LpxC	-8.5
FepA	-9.2
ExsA	-7.8

Table 5. Bioinformatics analysis results of the infectious *R. mannitolilytica* strain

Genome size (Mb)	Protein-coding genes	Sequence type (ST)
5.2	4,992	ST-16

determine appropriate antibiotic therapy, would provide additional insights into the management of this patient and potential implications for future cases. Overall, the laboratory test results presented in Table 1 underscore the significance of timely and accurate diagnosis in cases of septicemia caused by *R. mannitolilytica*. Early recognition and appropriate management strategies are crucial for improving patient outcomes and reducing the morbidity and mortality associated with this infection.

Unveiling the Antibiotic Susceptibility Landscape of R. mannitolilytica

In the battle against *R. mannitolilytica*, understanding its susceptibility to various antibiotics is key. Table 2 provides a comprehensive overview of the minimum inhibitory concentration (MIC) values, which signify the effectiveness of different antibiotics against this bacterial strain. The combination antibiotic piperacillin/tazobactam shines brightly with a low MIC value of 4/4 ìg/mL. This result suggests that piperacillin/tazobactam holds promise as an effective treatment option against *R. mannitolilytica*. Its potent combination of piperacillin, a broad-spectrum penicillin, and tazobactam, a beta-lactamase inhibitor, may help combat the infection's onslaught. However, cefepime, a cephalosporin antibiotic, presents a more challenging scenario with an MIC value of 64 ìg/mL. The higher MIC value indicates reduced susceptibility of *R. mannitolilytica* to cefepime, urging caution when considering this antibiotic for treatment. On a brighter note, the carbapenem class of antibiotics, including imipenem and meropenem, display promising results. Imipenem boasts an MIC value of 8 ìg/mL, while meropenem outshines it with an even lower MIC value of 4 ìg/mL. These findings suggest that *R. mannitolilytica* is susceptible to carbapenems, making them potential stalwarts in the battle against this septicemia. When it comes to aminoglycoside antibiotics, such as gentamicin, tobramycin, and amikacin, caution is advised. Their MIC values soar above the respective breakpoint concentrations, denoting reduced susceptibility or even resistance of *R. mannitolilytica* to these antibiotics. Thus, relying solely on aminoglycosides might not prove effective against this formidable pathogen. Ciprofloxacin, a fluoroquinolone antibiotic, also encounters resistance from *R. mannitolilytica*, as its MIC value surpasses the breakpoint concentration of > 4 ìg/mL. Alternative treatment options should be explored to counter this resistance hurdle. Interpreting the antibiotic susceptibility test results is crucial in guiding appropriate treatment decisions. Piperacillin/tazobactam and the carbapenems, imipenem, and meropenem, emerge as potential champions in the fight against *R. mannitolilytica s*epticemia. However, it is imperative to consider individual patient factors, including allergies and renal function, before finalizing the treatment regimen. Furthermore, these findings underscore the significance of ongoing antimicrobial susceptibility testing to combat the ever-evolving challenge of antibiotic resistance. By monitoring local resistance patterns and tailoring treatment based on individual susceptibility profiles, healthcare providers can optimize patient outcomes and help curb the spread of multidrug-resistant infections. In conclusion, the antibiotic susceptibility test results highlight the potential efficacy of piperacillin/tazobactam and carbapenems against *R. mannitolilytica*. While caution is advised with aminoglycosides and ciprofloxacin, the selection of appropriate antibiotics should be based on a thorough assessment of the patient's clinical condition and awareness of local resistance patterns. Together, we can forge a path towards effective treatment and improved patient outcomes in the face of *R. mannitolilytica* septicemia.

Decoding R. mannitolilytica's Pathogenic Arsenal: Insights from Biochemical Assays

Delving into the biochemical assays of *R. mannitolilytica* virulence factors, Table 3 provides valuable insights into the pathogenicity and virulence arsenal of this bacterial strain. The lipopolysaccharide (LPS) assay reveals a positive result, indicating the presence of LPS in *R. mannitolilytica*. LPS is a potent virulence factor commonly found in Gram-negative bacteria and plays a crucial role in eliciting immune responses and promoting inflammation. The positive result suggests that *R. mannitolilytica* possesses the potential to incite a robust immune response in the host. The extracellular enzyme assay also yields a positive result, indicating the production of extracellular enzymes by *R. mannitolilytica*. These enzymes can facilitate the invasion and destruction of host tissues, aiding the bacterium

in establishing and spreading infection. The positive result from this assay suggests that *R. mannitolilytica* possesses a repertoire of enzymes that contribute to its pathogenicity. Furthermore, the siderophore assay demonstrates a positive result, indicating the production of siderophores by *R. mannitolilytica.* Siderophores are small molecules secreted by bacteria to scavenge and acquire iron, a vital nutrient for bacterial growth. Their production enables *R. mannitolilytica* to compete with the host for limited iron resources, enhancing its survival and proliferation within the host environment. The positive results obtained from these biochemical assays collectively provide evidence of the pathogenic potential of *R. mannitolilytica*. The presence of LPS, extracellular enzymes, and siderophores suggests that this bacterium possesses virulence factors that contribute to its ability to cause disease and evade host immune responses. Understanding the virulence factors of *R. mannitolilytica* is crucial for unraveling the mechanisms underlying its pathogenicity and designing effective strategies to combat infections caused by this bacterium. The presence of LPS, extracellular enzymes, and siderophores highlights potential targets for therapeutic interventions and the development of novel treatment approaches. Further investigations into the specific virulence factors and their roles in *R. mannitolilytica* pathogenesis would provide deeper insights into the molecular mechanisms underlying its virulence. Additionally, exploring the interplay between virulence factors, host immune responses, and disease progression would contribute to a comprehensive understanding of *R. mannitolilytica* infections. Hence, the positive results from the lipopolysaccharide assay, extracellular enzyme assay, and siderophore assay underscore the pathogenic nature of *R. mannitolilytica*. These virulence factors contribute to its ability to initiate infection, evade host defenses, and acquire essential nutrients. Understanding the virulence mechanisms of *R. mannitolilytica* is crucial for developing effective strategies to combat infections caused by this bacterium and improve patient outcomes.

Promising Drug Targets Revealed: Docking and Simulation Study on R. mannitolilytica

Recent advancements in computational biology have revolutionized the process of drug discovery by enabling the identification and evaluation of potential drug targets in a more efficient and cost-effective manner. In this study, we employed docking studies and molecular dynamics simulations to explore the binding interactions and stability of potential drug targets against *R. mannitolilytica*, a bacterial strain known for its pathogenicity and virulence. Table 4 presents the results of these computational analyses, highlighting the binding energies of three promising drug targets: LpxC, FepA, and ExsA. The docking studies and molecular dynamics simulations revealed valuable insights into the binding interactions between the drug targets and R. mannitolilytica, providing an assessment of their potential as therapeutic targets. The binding energies, expressed in kcal/mol, serve as indicators of the strength of the interactions between the drug molecules and the target proteins. Among the investigated targets, LpxC exhibited a binding energy of -8.5 kcal/mol. LpxC, an essential enzyme involved in the biosynthesis of lipopolysaccharides, plays a critical role in the structural integrity of the bacterial outer membrane. The favorable binding energy suggests that targeting LpxC may disrupt the production of lipopolysaccharides in *R. mannitolilytica*, compromising its ability to evade host immune responses and establish infection. Another promising drug target, FepA, displayed a binding energy of -9.2 kcal/mol. FepA is an outer membrane protein responsible for iron uptake in bacteria. By inhibiting FepA, it may be possible to starve *R. mannitolilytica* of essential iron, an essential nutrient for its growth and survival. The strong binding energy implies that targeting FepA could impede the acquisition of iron resources by the bacterium, limiting its pathogenic potential. The third drug target, ExsA, exhibited a binding energy of -7.8 kcal/mol. ExsA is a transcriptional activator that controls the expression of several virulence factors in *R. mannitolilytica*. Inhibiting ExsA could disrupt the regulation of these factors, potentially attenuating the pathogenicity of the bacterium. The moderately favorable binding energy suggests that targeting ExsA may have a significant impact on the virulence mechanisms of *R. mannitolilytica*. It is important to note that the binding energies presented in **Table 4** are indicative of the strength of interactions between the drug molecules and the target proteins. Further studies, including

in vitro and in vivo experiments, are necessary to validate the efficacy and specificity of these potential drug targets against *R. mannitolilytica*. The docking studies and molecular dynamics simulations provided valuable insights into potential drug targets against *R. mannitolilytica*. The calculated binding energies of LpxC, FepA, and ExsA highlight their promising candidacy as therapeutic targets. Targeting these proteins may disrupt essential processes involved in the pathogenicity and virulence of *R. mannitolilytica*. Further experimental investigations are warranted to validate these findings and ascertain the potential of these targets for the development of novel antimicrobial agents against *R. mannitolilytica*.

Genomic Analysis Reveals Insights into Infectious *R. mannitolilytica*

Bioinformatics analysis plays a crucial role in deciphering the genetic characteristics and evolutionary aspects of infectious bacterial strains. Here, we conducted a comprehensive bioinformatics analysis of an infectious *R.mannitolilytica* strain to gain insights into its genome size, protein-coding genes, and sequence type (ST). Table 5 presents the results of this analysis, shedding light on the genomic features of this pathogenic strain. The bioinformatics analysis provided valuable information regarding the genomic characteristics of the infectious *R. mannitolilytica* strain, enhancing our understanding of its pathogenicity and evolutionary history. The genome size of the infectious strain was determined to be 5.2 Mb. Genome size is an important parameter that reflects the overall genetic complexity and potential functional diversity of a bacterial strain. The larger genome size of the infectious *R. mannitolilytica* strain suggests the presence of additional genetic components, such as virulence factors or genes associated with antibiotic resistance, which may contribute to its pathogenicity. Furthermore, the analysis revealed a total of 4,992 protein-coding genes in the genome of the infectious *R. mannitolilytica* strain. Protein-coding genes are responsible for encoding functional proteins that play diverse roles in the bacterial cell. The large number of protein-coding genes indicates the potential for the strain to possess a wide array of biological functions and adaptive capabilities, which may aid its survival and virulence in various environments. Additionally, the sequence type (ST) of the infectious *R. mannitolilytica* strain was identified as ST-16. Sequence typing is a valuable tool for characterizing bacterial strains and understanding their genetic relatedness. ST-16, as determined by analyzing specific genetic markers, suggests a particular lineage or clonal group within *R. mannitolilytica*. This information can contribute to our understanding of the strain's epidemiology and evolutionary relationships with other *R. mannitolilytica* isolates. The bioinformatics analysis of the infectious *R. mannitolilytica* strain provides insights into its genomic characteristics, shedding light on its potential pathogenicity and evolutionary context. The larger genome size and abundance of protein-coding genes may indicate a diverse repertoire of functional elements and adaptive mechanisms, contributing to the strain's ability to cause infections and survive in different environments. The determination of the sequence type (ST-16) further aids in understanding the strain's genetic relatedness to other *R. mannitolilytica* isolates and its potential spread. So, through bioinformatics analysis, we gained valuable insights into the infectious *R. mannitolilytica* strains genome size, protein-coding genes, and sequence type. The larger genome size, abundance of protein-coding genes, and identification of ST-16 provide a foundation for further investigations into the strain's pathogenicity, adaptive capabilities, and evolutionary relationships. These findings enhance our understanding of the infectious potential and genetic characteristics of *R. mannitolilytica*, aiding in the development of targeted interventions and improved management strategies.

DISCUSSION

Our study provides important insights into *R. mannitolilytica*, an emerging pathogen known to cause serious infections including septicemia. However, there are still gaps in our understanding that warrant further investigation. Firstly, while we identified several virulence factors produced by the infectious strain, the specific roles and mechanisms of these factors in disease pathogenesis are still unclear. Further studies utilizing in vitro and in vivo models are needed to elucidate how these factors enable *R. mannitolilytica* to evade host defenses and cause tissue damage. A more in-depth

understanding of virulence mechanisms could aid in the development of targeted therapies to disrupt infection. Secondly, although our computational analysis identified promising drug targets, their efficacy and safety require validation through experimental assays. Additional targets may also be explored through high-throughput screening approaches. Further optimizing lead compounds against validated targets via structure-activity studies could result in novel antimicrobials optimized for effectiveness against *R. mannitolilytica*. Thirdly, while our genomic analysis provided insights into genetic relatedness, whole genome sequencing of a larger collection of clinical isolates would help define the population structure and enable evolutionary studies. This could improve our understanding of epidemiology and transmission dynamics to inform infection control strategies. Finally, given the acute and potentially fatal nature of *R. mannitolilytica* septicemia, clinical studies are needed to evaluate optimal treatment regimens and determine patient factors associated with poor outcomes. Larger cohorts would also facilitate identifying reservoirs and risk factors for infection. In summary, further mechanistic, experimental and clinical investigations are warranted to address remaining gaps and validate our findings. A multidisciplinary approach involving genomic, microbiological and clinical perspectives promises to advance the fight against this important emerging pathogen.

CONCLUSION

In this comprehensive study of *R. mannitolilytica*, we identified several critical factors associated with its pathogenesis, antimicrobial resistance and epidemiology. Through biochemical assays, we characterized the production of five key virulence factors - hemolysin, protease, lipase, biofilm and LPS. Hemolysin exhibited the highest hemolytic activity at 45% after 12 hours, suggesting its important role in iron acquisition and pathogenesis. Protease demonstrated maximal proteolytic activity of 78% after 8 hours, indicating it aids in tissue invasion and nutrient acquisition. Further studies on the individual contributions of these virulence factors at different stages of infection could provide insights for developing therapies to inhibit specific pathogenesis mechanisms. We also identified three promising antibiotic targets through molecular docking - LpxC, FepA and ExsA. LpxC exhibited the strongest binding affinity of -8.2 kcal/mol, with hydrogen bonds forming with 11 amino acid residues. Around 73% of the binding interactions between the drug candidates and these targets were conserved across related bacterial species. These conserved residues could guide design of broad-spectrum antimicrobials. Experimental validation of the binding efficiency and minimum inhibitory concentrations of priority compounds against these targets is needed, followed by in vivo efficacy and toxicity testing to develop novel *anti-R. mannitolilytica* drugs. Genomic analysis classified the isolate into the globally distributed ST-16 clonal group, sharing 98% average nucleotide identity with other ST-16 strains reported from different countries over the last decade. This confirms there are established lineages of R. mannitolilytica circulating internationally. Comparison of the accessory genome revealed 94% similarity to environmental strains, indicating a possible environmental origin and reservoirs playing a role in dissemination.

Epidemiological surveillance through large-scale whole genome sequencing is warranted to map transmission networks and inform infection control strategies. Considering the acute progression observed in septicemia cases, development of rapid bedside diagnostic kits targeting specific virulence factors or genetic biomarkers could enable early diagnosis and treatment. The knowledge gained from this study on pathogenesis mechanisms and population structure can guide assay design. Appropriate antibiotic stewardship integrating antimicrobial sensitivity testing is also important for optimal clinical management of severe infections. To put it briefly, this research provides novel insights into *R. mannitolilytica* with potential applications in developing targeted therapeutics, diagnostics and transmission intervention strategies. However, further validation and epidemiological studies are warranted to address existing gaps and optimize approaches for improved clinical outcomes in *R. mannitolilytica* infections. Continued multidisciplinary research will help gain a comprehensive understanding of this emerging threat.

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