Virulence and resistance factors associated with the specie of *Proteus vulgaris*

ABSTRACT

Proteus vulgaris belongs to the Enterobacteriaceae family, colonizing mainly the gastrointestinal tract of animals, including humans. However, when there is some deregulation in the host's immune system, this pathogen spreads, causing several clinical manifestations. Currently in the literature little is discussed about the virulence and resistance profile of this species. Thus, the study aimed, through a literature review, to describe the main virulence factors and resistance of this strain. Studies have shown that *P. vulgaris* is associated with most Urinary Tract Infections (UTIs), despite being less isolated from *P. mirabilis*, exhibiting high motility ability related to its fimbriae, enzyme and endotoxin secretion, ability to produce urease and biofilm formation. As for resistance, the pathogen is intrinsically resistant to several antibiotics, requiring further studies for the development of new therapeutic alternatives.

Keywords: Infections; Proteus vulgaris; Resistance; Virulence.

1. INTRODUCTION

The genus *Proteus* spp., belongs to the Enterobacteriaceae family, characterized as facultative anaerobic Gram-negative bacilli, having five species well described taxonomically: *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri*, *Proteus hauseri* and *Proteus myxofaciens* [1]. These are part of the enteric microbiota of animals, regardless of cold-blooded or warm-blooded species, including humans, in addition to being found in contaminated water and food. Among the species, *P. mirabilis* and *P. vulgaris* stand out, as they are clinically relevant due to their pathogenicity [2].

P. mirabilis and *P. vulgaris* have the ability to colonize and cause infectious processes in humans, being characterized as opportunistic microorganisms. Such pathogens make use of conditions that make the individual's immunity vulnerable to cause various manifestations, from a simple urinary tract infection to more serious infections, such as meningitis and generalized infections [3]. Several factors contribute to the pathogenicity of these species, facilitating the onset of infection and the establishment of the microorganism at the site of infection.

Thus, the present study aimed to analyze the literature regarding the main characteristics of the bacterium *P. vulgaris*, as well as to investigate the main virulence and resistance factors involved with the microorganism.

2. MATERIALS AND METHOD

The information was obtained through data collection carried out by bibliographic survey, where platforms such as Google Scholar (<https://scholar.google.com.br>), PUBMED (<https://pubmed.ncbi.nlm. nih.gov/>), SciElo (<https://www.scielo.org/>), Science Direct (<https://www.sciencedirect.com/>), Scopus® (<https://www. scopus.com/>), LILACS (<https://lilacs.bvsalud.org/>) and BDTD (<https://bdtd.ibict.br/>). As an inclusion criterion, we considered articles published in the previously mentioned journals between the period of 2013 to 2023, which addressed the theme in question, and which were in Portuguese and English.

3. DEVELOPMENT

3.1 General enterobacteria and epidemiology of *P. vulgaris*

Proteus vulgaris is a short rod-shaped Gram-negative opportunistic enterobacterium, 0.4-0.8 µm in diameter and 1.0-3.0 µm in length, has motility through peritrichous flagella, is lactose and lysine decarboxylase negative and have the ability to hydrolyze urea [2]. It is commonly found colonizing the gastrointestinal tract of animals, forming part of the normal microbiota, and in food and water contaminated with waste [4]. Its pathogenic form is mainly associated with urinary tract infections (UTI), being responsible for more severe forms with formation of kidney stones [3]. However, this is also related to more serious conditions such as meningitis, wound infection, rheumatoid arthritis and septicemia in patients with burns [5].

In a study carried out by scientists, the isolation of 11 strains identified as *P. vulgaris* was verified, eight from manure, two from animals (dog) and only 1 from human origin (nasal mucosa) [5]. Having already investigated the bacteria that cause urinary infections, it was reported that of the 136 isolates at the Center for Clinical Analysis Diagnostics between the years 2017-2018, 4.5% (6 strains) were identified as *P. vulgaris* among female patients and males ranging in age from 3 to 89 years [6]. Furthermore, researchers point out that the main agents isolated from the urine of domestic animals and humans are *Escherichia coli* (37.64%), *Staphylococcus* spp. (19.66%) and *Proteus* spp. (13.48%) [7]. Reinforced by studies indicating *E. coli* and *Proteus* sp. as the main causes of UTIs [8].

Analyzes also reported the isolation of *P. vulgaris* from UTIs in patients treated at a metropolitan hospital, it was seen that *P. vulgaris* and *P. mirabilis* per se corresponded to 5.63% of isolations in patients aged 0 to > 60 years of age [9]. However, what has been observed is that despite its pathogenicity, *P. vulgaris* is still less isolated than *P. mirabilis*. In studies, 73 strains were isolated from the urine culture of domestic animals, of which 94.51% (64 strains) corresponded to *P. mirabilis* and only 5.49% (4 strains) were identified as *P. vulgaris* [10]. Even so, despite this difference, *P. vulgaris* has several virulence factors that contribute to its pathogenicity. In addition, it is important to emphasize that the high number of isolates from domestic animals alert us to a public health problem due to the high ease of transmission to humans, especially those with impaired immune systems, children and the elderly [11].

3.2 Virulence Factors

Several conditions favor the transition from the commensal to the pathogenic form, facilitating the onset of infection and the establishment of the pathogen, from factors that are in the cell structure itself, such as fimbriae and flagella, to the secretion of enzymes and endotoxins [4]. The simultaneous expression of different types of fimbriae is directly associated with the ability of the microorganism to adhere to different surfaces, including epithelial cells. These structures are proteinaceous, filamentous with high specificity for cell-cell and bacteria-host adhesion [12]. In addition, the mobility of this species in cycles by peritrichous flagella helps in the dissemination of the pathogen at the site of infection [12-13].

Thus, *Proteus vulgaris* exhibits the ability to swarm due to its high mobility. Studies point out that this bacterium can trigger the differentiation between cells with short rods to extremely elongated swarm cells with many flagella, which facilitates their displacement and dissemination on surfaces [14].

Furthermore, the presence of LPS (Lipopolysaccharide) represents great virulence in Gram-negative bacteria, including *P. vulgaris*, responsible for stimulating the action of prostaglandins and pro-inflammatory cytokines that can result in toxic shock syndromes [10]. Another characteristic of this species is the production of hemolysin, responsible for forming pores in eukaryotic membranes and the action of an amino acid deaminase that acts as a siderophore due to its affinity and capture of the iron ion, which is essential for the metabolism of *P. vulgaris* [15].

Another virulence factor that has been much discussed in urinary tract infections due to the dissemination of *P. vulgaris* is its ability to produce urease, causing the formation of stones [14]. This is due to the hydrolysis of urea into ammonia and CO^2, changing the pH of the urine and causing the precipitation of ions with consequent formation of stones [15]. These, in turn, obstruct the normal flow of urine, in addition to the high concentrations of urea being extremely harmful to the urinary epithelium [16]. *Proteus* urolithiasis is difficult to treat, given that the bacteria can be located inside the calculus, where it keeps multiplying and can easily spread to other organs [12].

As in other bacteria, biofilm formation also configures an important virulence factor, favoring the permanence of the pathogen at the site of infection and making it difficult to eradicate the microorganism. These microbial communities are recognized for becoming up to 1000 times more resistant when compared to their planktonic form, because the microorganisms are surrounded by an exopolymeric matrix composed of polysaccharides, proteins, extracellular DNA and lipids, which hinders the action of the cells of the immune system and the introduction of antibiotics [17].

Biofilms are formed through multiple stages, from bacterial adhesion to biotic and abiotic surfaces, through attachment, formation of small colonies, production of exopolymer matrix, to the mature biofilm stage, where cells that are in the upper layers begin to come off to colonize other surfaces [17]. In the case of *Proteus*, biofilms are formed from the initial adhesion of adhesins present in the fimbriae, resulting in crystalline structures that are mainly associated with obstruction of urinary catheters [18].

3.3 Resistance

Several studies have shown the resistance profile of *P. vulgaris* to some antibiotics, pointing to the urgent need for new therapeutic alternatives that can contribute to the control of these infections. According to the Clinical & Laboratory Standards Institute, *Proteus vulgaris* presents intrinsic resistance to some antibiotics, among which are: ampicillin, cephalosporin I – cefazolin and cefotetan, cephalosporin II – cefuroxime, tetracyclines – tigecycline, nitrofurantoin, polymyxin B and colistin [19]. However, this feature is commonly found in bacterial genomes, without the influence of selective pressure exerted by antimicrobials, and is not even related to horizontal gene transfer [20].

Investigations demonstrated the isolation of 17 strains of *P. vulgaris* from urinary infections, of these, about 94% showed resistance to the antibiotics ampicillin, tigecycline and chloramphenicol, while 12% showed resistance to imipenem [4]. In addition to this, other studies also reported the resistance of this species, with approximately 70% of the isolates presenting this characteristic to nitrofurantoin [21]. However, in contrast to these findings, a study with isolates of *Proteus* spp. from domestic animals, showed high in vitro sensitivity to the antibiotics imipenem, norfloxacin, amikacin, levofloxacin, ceftriaxone, florfenicol, marbofloxacin and amoxicillin/clavulanic acid, suggesting the efficacy of carpabenemics, fluoroquinolones, aminoglycosides, 3rd generation cephalosporin and amphenicols, while 73 isolates showed resistance to novobiocin and sulfamethoxazole-trimetropine [10]. In general, the study indicates a percentage of resistance to multidrugs of 45.21% (33 strains). It is noteworthy that the high sensitivity profile is associated with the restricted use of these drugs in veterinary medicine, which is not reflected in human medicine [22].

Studies evaluating the resistance profile of *P. vulgaris* isolated from UTIs in a metropolitan hospital. It was seen that 100% of the isolates showed resistance to ampicillin, cefepime, fluoroquinolones ciprofloxacin and levofloxacin, nitrofurantoin and cephalothin and sensitivity to amikacin, cefoxitin, gentamicin, imipenem, norfloxacin, ceftriaxone, piperacillin-tazobactam and tobramycin. These findings suggest the importance of correctly identifying the microorganism and using the TSA (Antimicrobial Sensitivity Test), ensuring the best treatment targeting and preventing the development of more resistance [9].

4. CONCLUSION

Proteus vulgaris is one of the most important pathogens that cause Urinary Tract Infections, exhibiting several virulence factors that facilitate and condition its permanence in the site of infection, related to mobility, secretion of enzymes and endotoxins, in addition to biofilm formation. However, this species has intrinsic resistance to several antibiotics, which demonstrates the need for the development of new therapeutic alternatives. In general, the current literature does not have many studies regarding the pathogenicity of this species, which also reinforces the need for further epidemiological and clinical studies.

REFERENCES

1. KishanRaj, S., Sumitha, S., Tang, TH, Citartan, M., & Chinni, SV (2021). Identification and comparative genomic characterization of npcRNA homologues in *Proteus vulgaris*. *Journal of biosciences* , *46* , 1-10.

2. Ribeiro, MG, Zappa, V., Bolaños, CAD, de Paula, CL, Callefe, JLR, Alves, AC, ... & Melville, PA (2017). Index of multiple antimicrobial resistance, minimal inhibitory concentrations and extended-spectrum beta-lactamase producers of strains of *Proteus mirabilis* and *Proteus vulgaris* isolated from domestic animals with various clinical manifestations of infection. Seminar: Agricultural Sciences, *38* (2), 775-789.

3. Ronanki, S. P., Ramya, P., Babu, A. J., & Sreedevi, B. (2022). Multidrug resistant-Proteus mirabilis and *Proteus vulgaris* isolated from milk and meat samples in Tirupati, Andhra Pradesh: An emerging public health threat.

4. Bilal, S., Anam, S., Mahmood, T., Abdullah, RM, Nisar, S., Kalsoom, F., ... & Anjum, FR (2019). Antimicrobial profiling and molecular characterization of antibiotic resistant genes from *Proteus vulgaris* isolated from a tertiary hospital, Islamabad, Pakistan. Pakistan Journal of Pharmaceutical Sciences, *32* .

5. Serafim, MLRC. Identification and antimicrobial resistance profile of bacteria isolated from different samples from the controlled landfill in the city of Campos dos Goytacazes-RJ. 2013. Dissertation (Master in Animal Science), State University of Norte Fluminense, Campos dos Goytacazes, 2013.

6. Cabeço, A. L. B., & Colombo, T. E. (2019). Bacteria causing urinary tract infections and their antimicrobial resistance profile. *Journal of the Health Sciences Institute*, *37*(2), 113-8.

7. Furini, A. A. C., dos Santos Silva, B. T. O., Chiaparini, J., Ramos, M. P. S. C. M., Martins, E. A., Atique, T. S. C., ... & de Castro, K. F. (2013). EPIDEMIOLOGICAL ANALYSIS, IDENTIFICATION AND PROFILE OF SUSCEPTIBILITY TO ANTIMICROBIALS OF ISOLATES FROM DOGS WITH URINARY TRACT INFECTION. *Acta Veterinaria Brasilica*, *7*(4), 288-293.

8. Marques, C., Belas, A., Aboim, C., Trigueiro, G., Cavaco-Silva, P., Gama, L. T., & Pomba, C. (2019). Clonal relatedness of *Proteus mirabilis* strains causing urinary tract infections in companion animals and humans. *Veterinary microbiology*, *228*, 77-82.

9. Araujo, K. L., & Queiroz, A. C. D. (2012). Analysis of the profile of the causative agents of urinary tract infection and of the carrier patients treated at the Hospital e Maternidade Metropolitano-SP. *J Health Sci Inst*, *30*(1), 7-12.

10. Zappa, V. Multiple antimicrobial resistance index, minimum inhibitory concentration and extended spectrum beta-lactamases in strains of *Proteus mirabilis* and *Proteus vulgaris* isolated from different diseases in domestic animals. 2015. Thesis (Doctorate in Veterinary Medicine). Paulista State University, Botucatu, 2015.

11. Bahashwan, S. A., & El Shafey, H. M. (2013). Antimicrobial resistance patterns of Proteus isolates from clinical specimens. *European Scientific Journal*, *9*(27).

12. Quinn, P. J., Markey, B. K., Leonard, F. C., Hartigan, P., Fanning, S., & Fitzpatrick, E. (2011). *Veterinary microbiology and microbial disease*. John Wiley & Sons.

13. Beltrão, EMB. Analysis of the genetic profile of virulence, resistance and clonal relationship of clinical isolates of *Proteus mirabilis* carriers and non-carriers of the blaKPC and blaNDM gene from a hospital in Recife-PE. 2021. Thesis (Doctorate in Tropical Medicine). Federal University of Pernambuco, Recife, 2021.

14. Wasfi, R., Abdellatif, G. R., Elshishtawy, H. M., & Ashour, H. M. (2020). First-time characterization of viable but non-culturable *Proteus mirabilis*: Induction and resuscitation. *Journal of Cellular and Molecular Medicine*, *24*(5), 2791-2801.

15. Siqueira, A. K., Ribeiro, M. G., Salerno, T., Takahira, R. K., Lopes, M. D., Prestes, N. C., & Silva, A. V. (2008). Sensitivity and multidrug resistance profile in *Escherichia coli* strains isolated from urinary tract infection, pyometra and dog feces. Brazilian Archive of Veterinary Medicine and Animal Science, *60*, 1263-1266.

16. Malinovski, E. (2021). MOST FREQUENT BACTERIA IN URINARY TRACT INFECTIONS. *Revista Saúde e Meio Ambiente*, *12*(1), 121-134.

17. Wasfi, R., Hamed, S. M., Amer, M. A., & Fahmy, L. I. (2020). *Proteus mirabilis* biofilm: Development and therapeutic strategies. *Frontiers in cellular and infection microbiology*, *10*, 414.

18. Filipiak, A., Chrapek, M., Literacka, E., Wawszczak, M., Głuszek, S., Majchrzak, M., ... & Adamus-Białek, W. (2020). Pathogenic factors correlate with antimicrobial resistance among clinical *Proteus mirabilis* strains. *Frontiers in microbiology*, *11*, 579389.

19. CLSI, E. (2016). Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*, 19087-1898.

20. Cox, G., & Wright, G. D. (2013). Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. *International Journal of Medical Microbiology*, *303*(6-7), 287-292.

21. Lazm, A. M., Jebur, M. S., & Alomashi, G. B. (2018). Sequencing of HpmA Gene in *Proteus mirabilis* of UTIs among rheumatoid arthritis patients. *J. Pharm. Sci. Res*, *10*(2), 265-271.

22. Greene, C. E. (2012). Infectious Diseases-of the dog and cat. St Louis. *MO: Elsevier Saunders*.