Original Research Article

Frequency of Uropathogenic Bacteria in Patients who came for Consultation to the National Reference University Hospital Center of N'Djamena (CHAD)

ABSTRACT

Background: Urinary tract infections are among the most common bacterial infections, both in urban medicine and in hospitals where nosocomial urinary tract infections rank first or second among the main sites of infection.

Aims: The general objective of this study is to determine the frequency of urinary tract infections and the main germs responsible for urinary tract infections in patients who came for consultation at the National Reference University Hospital of N'Djamena.

Methodology: This is a prospective cross-sectional study with non-probabilistic and consecutive sampling, conducted in the bacteriology unit of the National Reference University Hospital Center (NRUHC) in N'Djamena,between July and October 2023 and included all patients who had undergone a Cytobacteriological examination of urine.The identification of bacteria was based on morphological, cultural and biochemical characteristics.

Results: Of a total of 237 urine samples examined, 121 were positive with an overall frequency of 51% of which men represented 64% and women 36. The most affected age group was [59-69] years for 27 % of participants. The minimum age of positive cases was 4 years, the maximum age was 90 years and a mean age of 53.64 years. The bacteriological frequency was 73% of Uropathogenic Enterobacteria, 23% of Gram-positive cocci, 2% of Cocco Bacilli and 2% of strict aerobic Bacilli. The main bacteria isolated were *E. coli* (45%), *S. aureus* (12%), *Staphylococcus spp*. (10%), *K. pneumoniae* (9%), *Acinetobacter baumanii*(4%), *Raoultellaomithinolytica*(3%).

Conclusion: Many germs can cause urinary tract infections due to pathogenicity factors specific to each.

Keywords: frequency, Uropathogen, infection, Urine, Chad

1. INTRODUCTION

Urinary tract infections are among the most common bacterial infections, both in urban medicine and in hospitals where nosocomial urinary tract infections rank first or second among the main sites of infection [1].

These are common causes of consultation, generally leading to antibiotic treatment. They are mainly caused by Enterobacteria, whose resistance to antibiotics is increasingly common. The frequency of urinary tract infections is high, estimated at 150 million cases per year worldwide [2]. They are characterized by the presence of germs and leukocytes in the urine, and can develop on a healthy or pathological urinary tract. They can be acute or chronic, simple or complicated and lead to serious complications, including damage to renal function [1]. They affect both sexes and strike at any age. They appear in 20% of cases in

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Comment [U3]: 36%

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Comment [U6]: pathogens

men. On the other hand, they are the most common infections in women, 50% of women will suffer from at least one symptomatic episode during their lives [3].

Urinary tract infection covers various clinical realities: uncomplicated acute cystitis, asymptomatic bacteriuria, and even risk situations such as pyelonephritis, prostatitis and urethritis [4]. In order to avoid worsening or relapse, it is necessary to prescribe antibiotic therapy adapted to these urinary tract infections.Several germs are incriminated. Gram-negative bacilli are the most involved in these infections with a predominance of Enterobacteria. Other Bacteria (Gram-positive cocci) are also encountered in these infections [1]. The frequency of these Uropathogenic Bacteria is due to the presence of virulence factors, mainly adhesins, but also by their ubiquitous presence.

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Chad is one of the African countries that pays a heavy price for the endemic urinary tract infections. The health system is still in its infancy, especially in the case of the management of urinary tract infections. Indeed, this infection remains a major public health problem today. At the National Reference University Hospital of N'Djamena, very few studies on urinary tract infections have been carried out even though they are responsible for the increase in morbidity and mortality. This worrying situation led us to ask the following question:

The general objective of this study is to determine the frequency of urinary tract infections and the main germs responsible for urinary tract infections in patients who come for consultation at the National Reference University Hospital Center of N'Djamena.

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2. MATERIAL AND METHODS

2.1 Study framework

This prospective cross-sectional study was conducted at the bacteriology unit of the National Reference University Hospital Center (NRUHC) in N'Djamena, capital of the Republic of Chad, over a period of 3 months from July to October 2023.

2.2Study Materials

As biological material, we used the urine of patients who came for consultation at the NRUHC. The working material consisted of standard material, regularly used for manipulations in medical microbiology. The culture media used for our study were CLED medium, Muller Hinton (MH) and APIE 20E Gallery.

2.3Selection criteria and sampling

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The study included patients who went to the bacteriology laboratory for a urine culture and who consented to participate in the study. Patients who presented for a non-consenting urine culture were excluded. The sampling was non-probabilistic and consecutive. The minimum size of the representative sample was calculated by the following formula, dedicated to the calculation of frequency.

$$\frac{}{m^2}$$

Z = 95% confidence level (typical value of 1.96)
P = Prevalence of urinary tract infections (P = 0.1496) [5]
m = 5% margin of error (typical value of 0.05).

N = required sample size

Using this formula, our minimum sample size is: N ≥ 195 patients. Thus, our study population consisted of 237 people suspected of having urinary tract infection without distinction of age or sex, slightly higher than the minimum sample size initially determined.

2.4. Data Collection

Data collection was carried out using a questionnaire specifically designed for this purpose. Upon arrival at the laboratory, patients were interviewed in order to perform the CEU. Information on sociodemographic characteristics (age, sex, marital status, occupation, religion and place of residence) was recorded using this questionnaire.

Urine samples were collected early in the morning in a sterile container by the patients themselves or their parents or by health personnel and immediately sent to the Microbiology Laboratory for the Urine Cytobacteriological Examination.

Each urine sample was accompanied by an examination report and upon arrival at the laboratory, the patient's name, first name, age, date and registration number are then recorded.

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2.5. Cytobacteriological Examinations of Urine

Classical Methods of Cytobacteriological Examination of Urine (CEU) were used for each sample: macroscopic examination, microscopic examination, culture and complete identification.

2.5.1 Macroscopic Examination

This examination allowed to note the changes in physical characteristics of the urine. Thus, the following were noted: color, appearance, odor and foreign bodies.

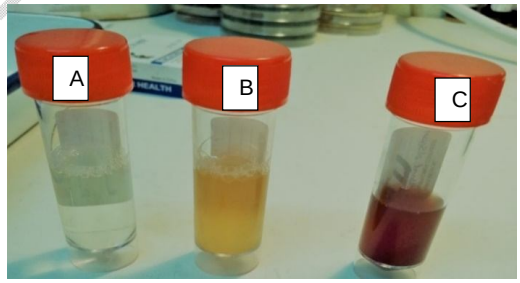

Fig. 1. The different aspects of Urine received for Analysis *(Photo Ayambi, 2023)*
(A=clear Urine, B=cloudy Urine and C=hematuric Urine)

2.5.2Microscopic Examination

Quantitative analysishasallowed to count the elements represented by unit of volume the cellular elements (leukocytes, red blood cells, epithelial cells, renal cells, or others), the microbial flora (bacilli or cocci, mycelial elements or yeasts), the crystals and the granular casts. For the leukocytes the average is calculated by several fields: 10^3 leukocytes/ml are the equivalent of 1-2 leukocytes/field.

Interpretation:
In physiological state: ≤ 10^3 leukocytes/ml and ≤ 10^3 red blood cells/ml

In case of urinary tract infection, in general: ≥ 10^4 leukocytes/ml, urinary tract bacteriosis ≥ 10^5 (± haematuria ≥ 10^4 red blood cells/ml)

The presence of vaginal cells indicates contamination of the urine: their presence should lead to caution in interpreting leukocyturia and/or hematuria, which may also be of genital origin.

Qualitative analysis allowed us to observe and assess the cells present in the sample (red blood cells, polymorphonuclear cells, crystals, and yeasts). Gram staining allowed us to suspect the presence of GRAM-negative bacilli and/or Gram-positive cocci.

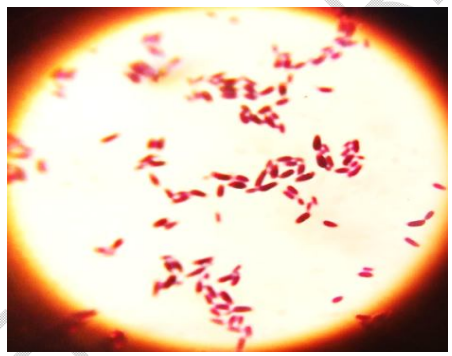

Fig. 2.Appearance of Gram-negative bacilli observed under the microscope after Gram staining *(Photo Ayambi, 2023)*

2.5.3. Cultivation

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Each sample was subjected to urine culture on CLED (Cystine Lactose Electrolyt Deficient) agar, which is a non-selective culture medium with enumeration of non-fastidious bacteria and Muller Hinton for identification by gallery.

The inoculation was carried out using the calibrated loop method. A drop of urine is taken vertically with the calibrated loop at 10 µl and by capillarity and inoculated by streaks on the agar plate: a central streak is inoculated then perpendicularly carry out an isolation from top to bottom of the plate by slightly loosening the last streaks. It consists of placing the inoculated plates in the incubator at 37 ° C for 18 to 24 hours.

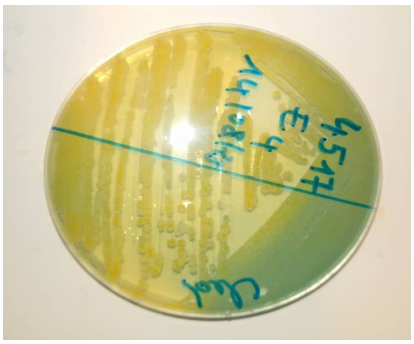

Fig. 3. Macroscopic appearance of *E. coli* colonies observed on CLED agar (*Photo Ayambi, 2023)*

Table 1 illustrates the conditions for interpreting the ECBU according to the significant criteria of urinary bacteriosis, type of microbial colony and Leukocyturia.

Table 1. Conditions for interpreting culture [6]

Stamm's significant criteria			Eventualities	Following
Leukocyturia	Bacterial urinary tract infections	Colony Types	Interpretation	Conduct
No	No	0	Sterile urine culture	Normal
Yes Leukocytes genitals	No	A kind	Antibiotic treatment Fastidious bacteria (BK)	To redo and adapt the techniques
No Contamination	Yes	A kind	Early infection Aplastic infection	Identification and ATB or to control
Yes	Yes	A kind	Typical infection	Identification and ATB
No	No	> 1	Defilement	None
Yes	No	> 2	Infection on probe?	To be checked
No	Yes	> 2	Defilement	None
Yes	Yes	> 2	Polymicrobial infection?	To do again

2.5.4. Identification

The identification of bacteria was done by the Gallery, techniques which consist of carrying out biochemical tests by a method specific to each family of microorganism.

Gallery API 20^{E} allowed the identification of Enterobacteria. The reading of these reactions was done using the Reading Table and the identification is obtained using the Analytical Catalogue.

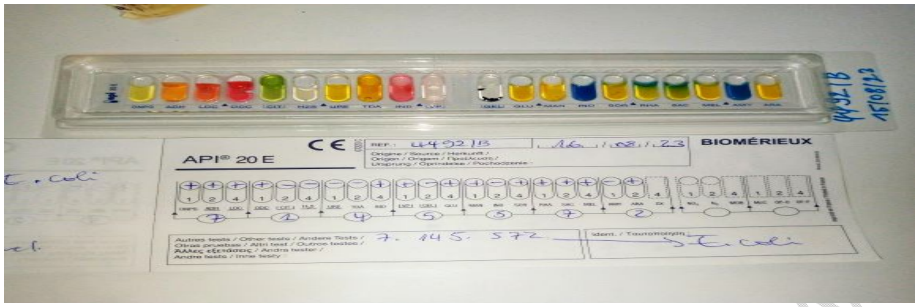

Fig. 4. *E. coli* species identified by Galerie API20^{E} (Photo Ayambi, 2023)

Identification of Gram-positive cocci using the Catalase Test:We used the bioMérieux ID color catalase reagent (dropper bottle containing a 10 volume hydrogen peroxide solution, a thickening agent and Evans blue). For *Staphylococcus species*, catalase is positive. On the other hand, for *Streptococcus species*, catalase is negative.

3. RESULTSAND DISCUSSION

3.1. Distribution of urine samples according to ECBU Results

Of 237 samples examined in our study, 121 (51%) were positive and 116 (49%) were negative. Figure 5 illustrates the overall frequency (51%) of Uropathogenic Bacteria found in our study at NRUHC.

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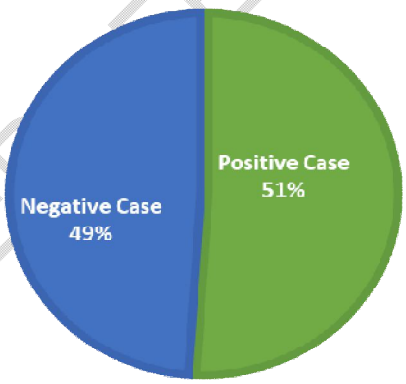

Fig. 5. Overall frequency of urinary tract infections

3.2. Distribution of samples according to sex

The gender distribution results are given in Figure 6. Of the 121 samples analyzed positive for bacterial culture, 78 were male with a frequency of 64 % (78/121) and 43 were female (36 %) with a sex ratio (M/F) of 1.81 in favor of males.

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Comment [U14]: 43 females (36%)

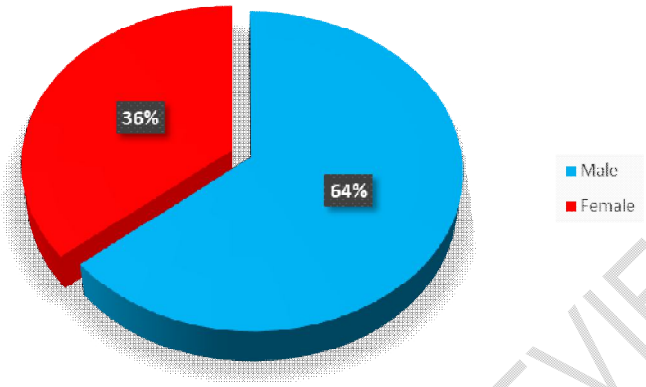

Fig. 6. Distribution of positive samples by gender

3.3. Distribution of results by age

Table 2 below shows the distribution by age group and it was observed that the most affected age groups were the age groups of [59-69] years with 27 % followed by the age groups of [48-58] years and [70-80] years all with 17 % of participants. The minimum age of positive cases was 4 years, the maximum age was 90 years and an average age of 53.64 years.

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Table 2. Distribution of UI frequency according to age groups

Age group	Number	Percentage (%)
[4-14]	6	5
[15-25]	9	7
[26-36]	12	10
[37-47]	12	10
[48-58]	21	17
[59-69]	33	27
[70-80]	21	17
[81-90]	7	6
Total	**121**	**100**

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3.4. Frequency distribution by profession

Figure 7 shows us the distribution of the frequency of urinary tract infections according to profession. It appears that the highest frequency is observed among traders with 31% followed by students with 29%.

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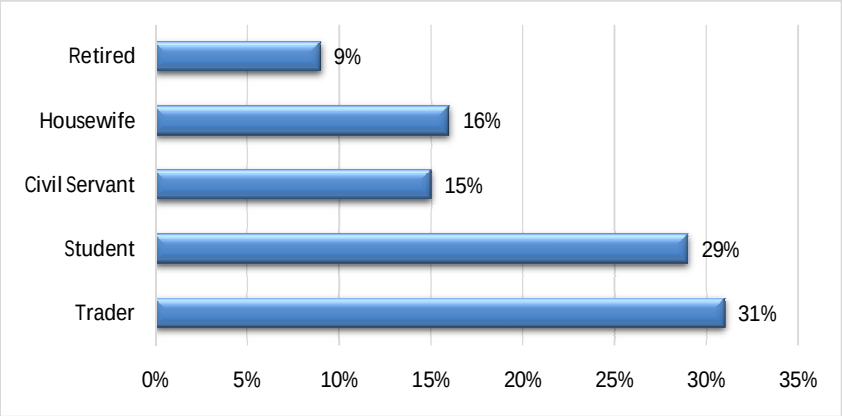

Fig. 7. Frequency distribution by profession

3.5. Bacteriological Results

According to the bacteriological results, we highlighted a frequency of 73% of Uropathogenic Enterobacteria with predominance of *E. coli* (45%), 23% of Gram-positive cocci, 2% of Cocco bacilli and 2% of strict aerobic bacilli. Figure 7 shows us the distribution of the biological profile of urinary tract infections.

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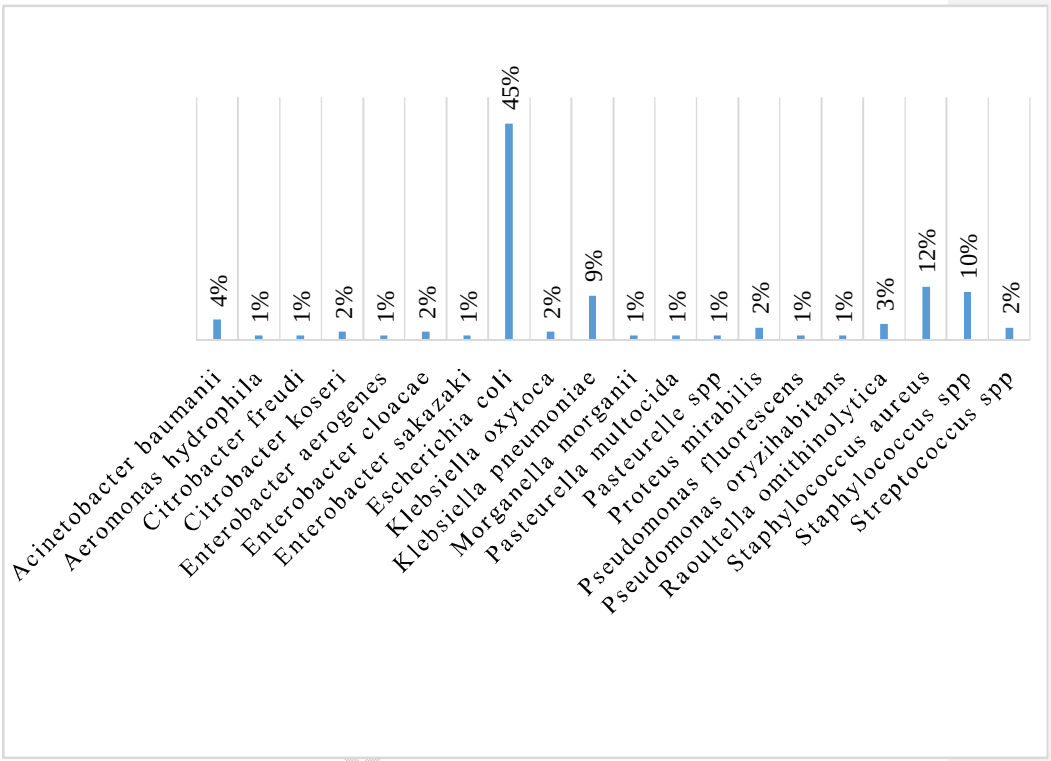

Figure 8. Frequency distribution according to isolated Uropathogenic Bacteria

3.6. Discussion

This cross-sectional study aimed to determine the frequency and antibiotic resistance profile of Uropathogenic Bacteria at the National Reference University Hospital of N'Djamena. Over a period of three months, 237 urine samples were examined by CEU, of which 121 tested positive for urinary tract infection, with a frequency of 51% with a male predominance of 64% compared to 36% in women, unlike most studies conducted in the field [7]. This frequency is significantly higher than that found in a study in Zinder in Niger where the authors found 26.59%, byagainst the result of the distribution according to sex agrees with ours [2]. A higher rateraised wasreported by a study conducted in Cameroon at the Laquintinie Hospital in Douala (68 %) in 2020 by [8]. In our study, the higher rate of urinary tract infections in men could be explained by the fact that the National Reference University Hospital Center of N'Djamena in which we carried out our sample collection and analysis is much more frequented by men than women, as well as by the fact that the request for CEU is more systematic in men given the risk factors for complications that are more significant in the latter. In addition, opposite this Hospital, there is a university hospital center for mothers and children, which is a structure specifically suited to the care of women and children. This means that few women come for a medical visit to our reception structure.

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The distribution of urinary tract infection according to the age of the patients shows that the frequency of this infection seems to increase with age and the most important group is that [59-69] with27% followed by those [48-58] and [70-80] all with 17%. These results are in agreement with the result of [9] in Algeria. These results could be explained by the fact that the factors involved in the increase in the incidence of urinary tract infections are multiple and bladder motility disorders (effect of medications, bed rest, etc.), dehydration, poor hygiene and reduced immune defenses increase with age. Overall, the urinary tract is poor in immunocompetent cells. This result confirms the literature according to which, in men, urinary tract infections increase with age, particularly in relation to prostate pathology.

The distribution of frequency according to profession shows that urinary tract infection is higher among traders (31%).and students (29%).Thisfrequent could be explainedby the level of education and social status of the latter. Indeed, most of the traders included in this study stopped their studies at the primary level and are therefore partly unaware of the hygienic conditions that could allow them to avoid this infection.

Bacteriologically, 121 samples tested positive for bacteriological culture out of 237 with a predominance of Enterobacteria (73%) followed by Gram-positive cocci (24%),coccobacilli (2%) and strict aerobic bacilli (2%) regardless of age and sex. The main bacterial species isolated were *E. coli* (45%), *Staphylococcus aureus* (11%), *Staphylococcus spp.* (10%), *Klebsiella pneumoniae (*8%) and *Acinetobacter baumanii*(4%). We note that this frequency is related to the pathophysiology of urinary tract infection which is generally ascending [2]**.** These results are consistent with those of the study conducted by Chekroud and Fathi in Algeria who reported that Enterobacteria represented 61% of the germs responsible for urinary tract infections [9]. This frequency can be explained by the existence of a strong colonization of the perineum by Enterobacteria of digestive origin, in particular *E. coli*. The higher frequency of *E. coli* could be explained by the specific factors of Uropathogenicity. *E. coli* has adhesins, capable of binding the bacteria to the urinary epithelium and preventing its elimination by bladder emptying. This is in agreement with the results of [2] and [10] who reported a frequency of *E. coli* at 51% and 67.53% respectively in a study conducted in Niger and Algeria. A frequency of 8%of UIKlebsiella *pneumoniae* has been observed. This frequency could be explained by the presence of several pathogenic and virulence factors in this species. Indeed, *Klebsiella pneumoniae* has a polysaccharide capsule which gives it a strong powerinvasive while protecting the bacteria from the host's immune defense. Aldioumareported 8% of urinary tract infections due to *Klebsiella pneumoniae* in 2002 in Bamako [11]**.**

We observe a frequency of UI due to *Staphylococcus aureus*at 11%. This frequency would be linked to the expression of virulence factors of this species. In *S. aureus, there are* surface proteins or adhesins that allow the bacteria to colonize the host but also factors that lead to the development and spread of the infection. This frequency is much higher than that found by [12] in a study conducted in Bamako in 2006 which was 4.64%. On the other hand, Diassanafound 34.28% of *Staphylococcus aureus* in 2000 in Bamako at the Point G national hospital [13].

4. CONCLUSION

This study, which aimed to determine the frequency and antibiotic resistance profile of Uropathogenic bacteria at the CHURN, made it possible to obtain, by analysis of the ECBU, an overall frequency of urinary tract infection of 51% with male predominance. This frequency seems to increase with age. Many germs can cause urinary tract infections due to pathogenicity factors specific to each**.** In our study, Enterobacteria were the most

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incriminated Uropathogens with predominance of *E. coli* followed by *Staphylococcus aureus* and *Klebsiellapneumoniae.*

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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