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ORIGINAL ARTICLE

Combating Biofilm: Melatonin's Role in Mitigating Multidrug-Resistant Uropathogens in Cancer

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Key words: ***Multidrug-resistant bacteria; Uropathogenic biofilms; Melatonin antibiofilm activity; Urinary tract infections (UTIs); infections in cancer patients***

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ABSTRACT

Background*: Multidrug-resistant (MDR) bacterial infections, particularly urinary tract infections (UTIs), are prevalent in cancer patients, due to long-term immunosuppression. Uropathogenic bacteria like Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, complicate treatment through biofilm formation, enhancing antibiotic resistance. Recent studies has explored the potential of melatonin, an antioxidant hormone, to disrupt bacterial biofilms.* ***Objective(s)****: This study analyzes the antibiotic resistance of uropathogenic bacteria from cancer patients and evaluates the efficacy of melatonin in inhibiting biofilm formation in these MDR strains.* ***Methodology****: 65 urine samples from cancer patients were collected at Sabha Oncology Center. Bacteria were identified using standard techniques, and antibiotic susceptibility was evaluated via the Kirby-Bauer method. Biofilm formation was measured using crystal violet staining, and melatonin's antibiofilm activity was tested from 10 µg/mL to 1000 µg/mL.* ***Results****: E. coli was the most prevalent pathogen (32.69%), followed by K. pneumoniae (17.30%) and P. aeruginosa (15.38%). All isolates exhibited high resistance to amoxicillin/clavulanic acid and cefotaxime with Strong biofilm. Melatonin notably inhibited E. coli biofilm formation at higher concentrations, with lesser effects on K. pneumoniae and P. aeruginosa.* ***Conclusion****: The prevalence of MDR uropathogens and their biofilms formation underscore the need for new therapies in cancer patients. Melatonin may serves as a complementary treatment to inhibit biofilm, particularly in E. coli. Additional research is required to assess its clinical application in managing biofilm-associated infections.*

INTRODUCTION

The rising prevalence of multidrug resistance bacterial infection in cancer patients poses a severe challenge to adequate medical treatment due to cancer treatment[1], including chemotherapy and radiation, which significantly increases the risk of opportunistic infections [2]. Urinary tract infections (UTIs) are common among individuals with weakened immune systems[3]. Due to the rise of multidrug- resistance bacteria and biofilm producers, UTIs have become a growing concern, particularly for those with weakened immune systems like cancer patients[3,4] The combination of weakened immune systems, aggressive cancer therapies, and the presence of resistant bacteria and biofilms can lead to persistent UTIs in cancer patients, complicating treatment[5] and leading to recurrent UTIs and potentially serious outcomes.[5,6]. The urgent need for new approaches to combat UTI in cancer patients, particularly in light of antibiotic resistance, has led to research into natural compounds such as Melatonin. Melatonin is an indoleamine produced by the pineal gland in response to darkness [7] and studies[9,10] indicate its role as a cellular protector, antioxidant, anticancer, and antitumor. Moreover, studies[8,9] have shown its activity against bacterial growth and in disrupting bacteria biofilms. However, there is still a lack of research specifically focusing on the efficacy of melatonin in biofilm inhibition among MDR uropathogens isolated from cancer patients. Given the increasing prevalence of MDR bacteria and biofilm-associated infections in this population, understanding the role of melatonin in combating these challenges could be crucial for improving patient outcomes. This study aims to analyze the antibiotic resistance patterns of bacteria causing UTIs in cancer patients and to evaluate the ability of melatonin to inhibit biofilm formation in highly biofilm-producing bacteria. The

research aims to improve treatment approaches for UTI in cancer patients by investigating these critical issues.

METHODOLOGY

Samples Collection

Sixty-five urine samples were collected from cancer patients attending the Sabha Oncology Center from September 2022, to December 2022. The samples were colleted from male and female patients of different age groups. Midstream urine samples were collected from all patients using sterile containers. Relevant patients information, including age, sex, chronic diseases, place of diagnosis, and treatment was recorded with the patients' consent. The samples were then transported for culture and diagnosis within 30 minutes of collection.

Culture and Identifications of Samples

Urine samples were collected aseptically and cultured on Brain Heart Infusion Broth to promote bacterial growth. After incubation (37°C, 48 hours), turbid samples were subcultured on MacConkey agar and CLED agar (24 hours, 37°C) to isolate colonies. Pure isolates were sub-cultured on Brain Heart Infusion agar and morphologically characterized. For identification, isolates underwent routine biochemical tests (lactose fermentation on MacConkey agar, oxidase, catalase) and automated systems (API 20E for Enterobacteriaceae, VITEK 2 Compact (BioMérieux, France) for broader identification, The VITEK 2 Compact system was used for automated identification and confirmation of bacterial isolates. Bacterial suspensions were prepared and loaded into the VITEK 2 cards, and the system provided identification and susceptibility results based on biochemical reactions and growth patterns.[10]

Antibiotics susceptibility Profile

Antibacterial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Scharlau, Spain) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (M100–S124, 2012)[12]. Bacterial suspensions were adjusted to a 0.5 McFarland standard prior to inoculation onto the agar plates. Antibiotic discs were placed on the agar surface, and the plates were incubated at 37°C for 18-24 hours. The antibiotics tested included Tetracycline (TE30), Ciprofloxacin (CIP5), Penicillin (PEN10), Amikacin (AK30), Chloramphenicol (CHL30), Amoxicillin/Clavulanate (AMC30), Nalidixic Acid (NAL30), Cefotaxime (CTX30), Ceftriaxone (CRO30), Gentamicin (CN30), Colistin (COL30), Nitrofurantoin (F300), Azithromycin (AZT30), Meropenem (MER10), Ertapenem (ERT10), and Imipenem (IMP10). After incubation, the diameters of inhibition zones around the discs were measured and interpreted based on (CLSI M100) standards to determine bacterial sensitivity or resistance to the tested antibiotics.

Biofilm Formation assay

Each pure isolate was inoculated into polystyrene test tubes containing 10 mL of Brain Heart Infusion broth (Scharlau, Spain) supplemented with 5% sucrose. The tubes were then incubated at 37°C for 48 hours. After incubation, the media was discarded, and the tubes were stained with 2 mL of crystal violet dye for 5 minutes. Subsequently, the tubes were rinsed three times with PBS (phosphate-buffered saline) solution and left to dry upside down. The biofilms formed on the inner surfaces of the tubes were then dissolved by adding 1 mL of absolute propanol. The absorbance of the resulting solution was measured using a spectrophotometer at a wavelength of 595 nm.[13]

Melatonin Biofilm Inhibition Assay:

A stock solution of melatonin (Sigma-Aldrich, Germany) at 1g/mL was prepared in 10% ethanol. From this, test concentrations of 400 µg/mL and 100 µg/mL were made which were further diluted to produce a range of working concentrations: 10 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, and 1000 µg/mL. The test concentrations were added to bacterial cultures in Brain Heart Infusion (Scharlau, Spain) supplemented with sucrose before inoculation and incubation. After incubation, results were measured following the previous established protocol.

Statistical Analysis

Statistical analyses were conducted using SPSS version 25. Descriptive statistics summarized bacterial species prevalence across cancer types and genders, calculating frequencies for each bacterial species. Overall prevalence was determined by dividing the number of cases by the total isolates. A Chi-square test assessed the relationship between bacterial species and cancer type or sex with significant set at $p<0.05$. Antibiotic resistance profiles analyzed across 16 antibiotics with average resistance reported for groups such as beta-lactams and fluoroquinolones. Descriptive statistics summarized biofilm formation capacity (OD595), and the effect of melatonin on biofilm inhibition. Results were expressed as frequencies (n), percentages (%), and means with standard deviations (± SD) for continuous variables.

RESULTS

The bacterial distribution in different cancer types was summarized in Table 1. A total of 52 cases were documented, involving a range of bacterial species across different cancer types, with *E.coli* being the most common species. Notably, it was identified in 17 cases, accounting for 32.69% of the total cases , and was particularly common in breast cancer (n=6), colorectal (n=5), and prostate (n=2) cancers. *K.pneumoniae* was the second most common pathogens in 9 cases (17.30%), mainly in breast and prostate cancer patients. *P.aeruginosa* was present in 8 cases (15.38%), with a

broader distribution across breast, leukaemia, prostate, colorectal, and liver cancers. Less frequently observed bacteria included *S.ficaria* (11.53%) and *S.odorifera* (7.69%), both of which were mainly found in breast and colorectal cancers. on the other hand, several bacterial species were detected sporadically, such as *A.baumannii* and *B.cepacia*, which occurred exclusively in breast cancer. Certain cancer types, such as cervical and liver cancer, had minimal bacterial presence, with only single cases of *E.coli* and *P.aeruginosa* detected.

Table 1: Bacterial Prevalence Across Various Cancer Types

	Breast Cancer	Ceverical Cancer	Leukemia	Prostate Cancer	Colorectal Cancer	Blood Cancer	Pancreas Cancer	Liver Cancer	Total	Total Prevalence %
Acinetobacter baumannii	3	0	0	0	0	0	0	0	3	5.76
Burkholderia Cepcia	1	0	0	0	0	0	0	0	1	1.92
Escherichia coli	6	1	1	2	5	1	1	0	17	32.69
Klebsiella pneumoniae	5	0	0	3	1	0	0	0	9	17.30
Pseudomonas aeruginosa	2	1	2	1	1	0	0	1	8	15.38
Serratia ficaria	3	0	0	0	1	2	0	0	6	11.53
Serratia odorifera	4	0	0	0	0	0	0	0	4	7.69
chryseobacterium indologenes	0	1	0	0	0	0	0	0	1	1.92
Serratia plymuthica	0	0	1	0	0	0	0	0	1	1.92
Serratia rubidaea	0	0	0	1	0	0	0	0	1	1.92
Stenotrophomanas maltophila	0	0	0	0	1	0	0	0	1	1.92
Total	24	3	4	7	9	3	1	1	52	100

The distribution of bacterial isolates across genders is summarized in table 2, with *E.coli* being the most frequently isolated bacterium, accounting for 32.69% of the total cases (11 in females and 6 in males*). K.pneumoniae* , comprising 17.31% of the cases (6 in females and 3 in males), while *P.aeruginosa* was present in 15.38% of cases , with an even distribution between males and females. Other bacteria, such as *S.ficaria* (11.54%) and *S.odorifera* (7.69%), were primarily found in females, whereas *S.rubidaea*, *S.maltophilia*, and *C.indologenes* were present only in males. A Chi-Square Test for Independence (χ^2 = 14.997, p = 0.132, df = 10) indicated no statistically significant association between gender and bacterial type.

Table 2: Gender-Based Frequency and Overall Prevalence of Bacterial Species

Bacteria Type	Female	Male	Frequency	Frequency %
Acinetobacter baumannii	3	0	3	5.769231
Burkholderia Cepcia	1	0	1	1.923077
Escherichia coli	11	6	17	32.69231
Klebsiella pneumoniae	6	3	9	17.30769
Pseudomonas aeruginosa	4	4	8	15.38462
Serratia ficaria	6	0	6	11.53846
Serratia odorifera	4	0	4	7.692308
Serratia rubidaea	0	1	1	1.923077
Stenotrophomanas maltophila	0	1	1	1.923077
chryseobacterium indologenes	1	0	1	1.923077
serratia plymuthica	0	1	1	1.923077

A total of 12 bacterial species were isolated from the urine of cancer patients and tested for resistance against 16 antibiotics (Table 3). *S.odorifera* exhibited a 100% resistance to amoxicillin/clavulanic acid (AMC30) and cefotaxime (CTX30), and showed 50% resistance to penicillin (PEN10), amikacin (AK30), meropenem (MER10), ertapenem (ERT10), and imipenem (IMP10). Resistance to tetracycline (TE30) and nitrofurantoin

(F300) was 25% and 50%, respectively. *K. pneumoniae* displayed complete resistance (100%) to AMC30, CTX30, and PEN10, with lower resistance levels to tetracycline (TE30) at 40%, ciprofloxacin (CIP5) at 20%, and nitrofurantoin (F300) showing no resistance. *A.baumannii* exhibited 100% resistance to AMC30, CTX30, PEN10, chloramphenicol (CHL30), and nitrofurantoin (F300), while showing no resistance to most other antibiotics. *P.aeruginosa* had 100% resistance to AMC30, CTX30, and ceftriaxone (CRO30), with 50% resistance to penicillin (PEN10), amikacin (AK30), gentamicin (CN30), ertapenem (ERT10), meropenem (MER10), and imipenem (IMP10). *E. coli* demonstrated 100% resistance to AMC30 and CTX30, 66.66% resistance to tetracycline (TE30), ciprofloxacin (CIP5), and chloramphenicol (CHL30), while showing 16.66% resistance to nitrofurantoin (F300). *S. ficaria* displayed high resistance to AMC30, CTX30, and PEN10 at 100%, while tetracycline (TE30), ciprofloxacin (CIP5), and nitrofurantoin (F300) showed 33.33% resistance each. *C. indologenes* exhibited 100% resistance to AMC30 and CTX30, but was susceptible to most other antibiotics. *S. plymuthica* and *S. rubidaea* were both 100% resistant to AMC30, CTX30, and PEN10, with no significant resistance recorded to other antibiotics. *S.maltophila* demonstrated 100% resistance to AMC30, CTX30, ciprofloxacin (CIP5), and PEN10, but no resistance to nitrofurantoin (F300). *B. cepacia* displayed 100% resistance to penicillin, ceftriaxone, ciprofloxacin, and amoxicillin/clavulanic acid, but only 33.33% resistance to nitrofurantoin (F300).

Overall, the highest resistance rates across all isolates were observed for AMC30 and CTX30 at 100%, followed by PEN10 at 88.88%. Resistance was lowest for antibiotics like gentamicin (CN30) at 16.66%, aztreonam (AZT30) at 15.15%, and nitrofurantoin (F300) at 27.27%.

Table 3: Antibiotcs resistace freqancy among the isolated bacteria

	S. odorifera	*K. pneumoniae*	*A. baumannii*	*P. aeruginosa*	*E. coli*	*S. ficaria*	*C. indologenes*	*S. plymuthica*	*S. rubidaea*	*S. maltophila*	*B. Cepcia*	Total Resistance (%)
TE30	25	40	0	0	66.66	33.33	0	0	0	0	38.88	18.53
CIP5	0	20	0	0	66.66	33.33	0	0	0	100	27.77	22.52
PEN10	50	100	100	50	100	100	0	0	100	100	88.88	71.71
AK30	50	0	0	50	0	33.33	0	0	0	0	11.11	13.13
CHL30	25	20	100	0	66.66	33.33	0	0	0	0	44.44	26.31
AMC30	100	100	100	100	100	100	100	100	100	100	100	100
NAL30	75	40	0	50	66.66	33.33	0	0	0	0	50	28.63
CTX30	100	100	100	100	100	100	100	100	100	100	100	100
CRO30	50	0	100	100	0	0	0	0	0	0	22.22	24.74
CN30	0	0	0	50	0	33.33	0	0	0	100	0	16.66
COL30	0	20	0	0	66.66	33.33	0	0	0	100	27.77	22.52
F300	50	0	100	0	16.66	100	0	0	0	0	33.33	27.27
AZT30	50	0	0	100	0	0	0	0	0	0	16.66	15.15
MER10	50	0	0	50	0	33.33	0	0	0	0	11.11	13.13
ERT10	50	0	100	50	0	33.33	0	0	0	0	22.22	23.23
IMP10	50	0	0	50	0	33.33	100	100	0	0	11.11	31.31

The average resistance percentages across different antibiotic groups were analyzed (Table 4). Tetracyclines showed an average resistance of 18.53%, while the fluoroquinolones group exhibited a resistance rate of 22.52%. The beta-lactams (penicillins) group had one of the highest average resistance rates at 71.71%, whereas aminoglycosides demonstrated a significantly lower resistance average of 14.89%.

Resistance to chloramphenicol was noted at 26.31%, and the beta-lactams (penicillins with beta-lactamase inhibitors) group exhibited complete resistance at 100%. Quinolones showed a moderate resistance of 28.63%, while the beta-lactams (cephalosporins) group had an average resistance of 62.37%.

The polymyxins group presented with a resistance rate of 22.52%, and the phosphonic acids group, represented by nitrofurantoin, had an average resistance of 27.27%. Monobactams demonstrated a relatively low resistance rate at 15.15%, while carbapenems displayed an average resistance of 22.55%.

Table 4: Average Resistance Percentages Across Antibiotic Group

Antibiotic Group	Average Resistance (%)
Tetracyclines	18.53
Fluoroquinolones	22.52
Beta-lactams (Penicillins)	71.71
Aminoglycosides	14.89
Chloramphenicol	26.31
Beta-lactams (Penicillins with beta-lactamase inhibitor)	100
Quinolones	28.63
Beta-lactams (Cephalosporins)	62.37
Polymyxins	22.52
Phosphonic acids	27.27
Beta-lactams (Monobactams)	15.15
Beta-lactams (Carbapenems)	22.55

The ability of 12 bacterial strains to form biofilms was assessed, using the median optical density (OD595). *E.coli*, *K.pneumoniae*, and *P.aeruginosa* (Figure 1)demonstrated strong biofilm formation, each with a median OD595 above 1. *B.cepacia* and *A.baumannii* exhibited moderate biofilm formation, with OD595 values between 0.5 and 1. Other species, such as *C.indologenes*, *S.ficaria*, and *S.maltophila*, produced weak to moderate biofilms, with OD595 values below 0.5 but above the negative control. The remaining bacterial strains, including *S.odorifera*, *S.plymuthica*, and *S.rubidaea*, showed minimal biofilm formation, with OD595 values close to the negative control.

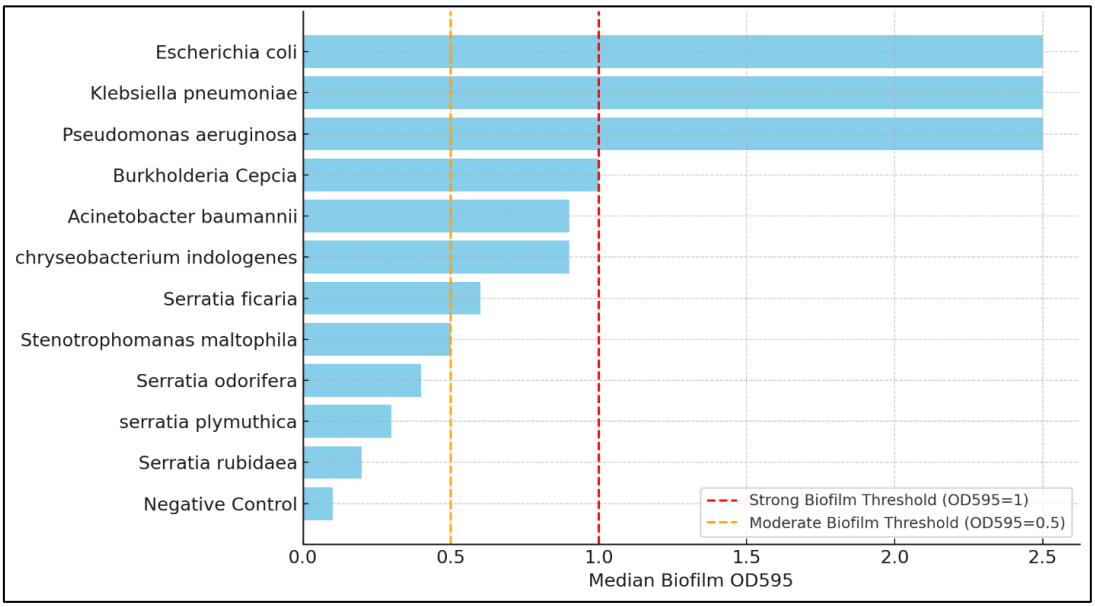

Fig. 1*:* Biofilm median formation among the isolated bacteria

The antibiofilm activity of melatonin was assessed against three potent biofilm-forming bacterial strains: *E.coli*, *K.pneumoniae*, and *P.aeruginosa*(Figure 2). The effectiveness of Melatonin was measured at concentrations ranging from 10 µg/ml to 1000 µg/mL, with biofilm inhibition evaluated by optical density (OD595).

E. coli showed an increasing reduction in biofilm formation as the melatonin concentration increased, with a substantial decrease at 1000 µg/ml, where biofilm formation dropped to an OD595 below 0.5. *P.aeruginosa* exhibited a more gradual reduction in biofilm formation, with melatonin maintaining a relatively stable OD595 above 2.0 at concentrations up to 400 µg/ml. However, at 1000 µg/ml, a noticeable decrease in biofilm formation was observed, dropping below 1.5. *K.pneumoniae* displayed the most consistent resistance to biofilm formation, with only a slight reduction from 2.5 to just below 2.0 across melatonin concentrations, with a sharp decrease occurring at the highest concentration of 1000 µg/ml.a

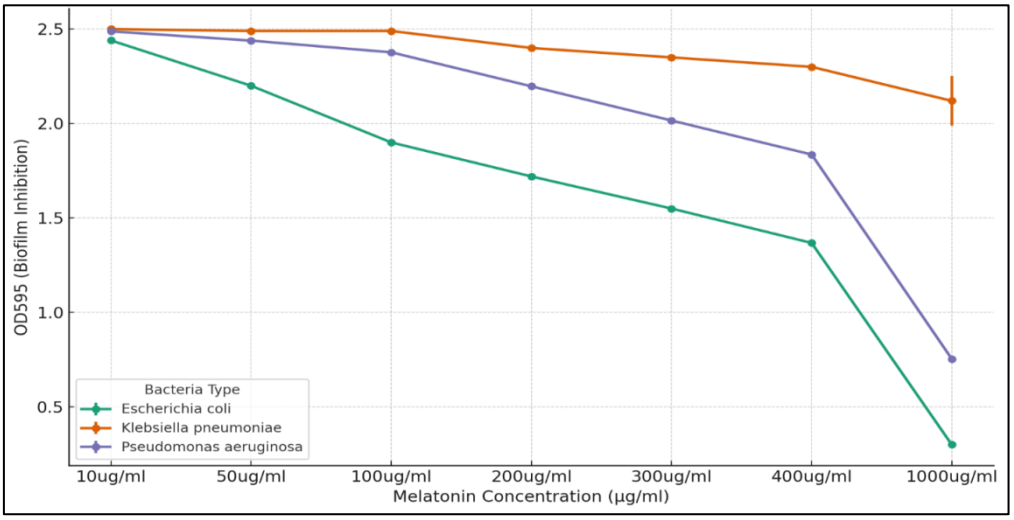

Fig. 2*:* Antibiofilm activity of Melatonin against strong biofilm former bacteria

DISCUSSION

The findings of our study provide new insights into the prevalence, distribution, and antibiotic resistance profiles of bacterial species isolated from cancer patients. Our results highlight the significant role that opportunistic bacterial infections play in complicating cancer treatment, particularly given the diversity of bacterial species and their variable resistance to antibiotics.

The most notable observation from our data set is the high prevalence of *E.coli*, which was the dominant species, accounting for 32.69% of all bacterial isolates. This finding is consistent with previous studies[14,15] that identified *E.coli* as a common pathogen in nosocomial and community-acquired infections, especially in immunocompromised individuals such as cancer patients. Interestingly, *E. coli* was predominantly isolated from breast, colorectal, and prostate cancer patients. The frequent detection of this organsim in breast cancer patients (n=6) suggests that these individuals may be more susceptible to urinary tract or bloodstream infections caused by *E. coli*, a concept that requires further investigation.

K.pneumoniae was the second most common organism , accounting for 17.30% of the isolates, and was observed in both breast and prostate cancer patients. Its widespread presence is of concern because *K.pneumoniae* is a well-known multidrug-resistant pathogen[16,17], and is often associated with serious infections in patients with compromised immune systems.

P.aeruginosa also has been widely implicated across several types of cancers, including breast, leukaemia, prostate, and colorectal cancers. Its ability to persist in diverse environments and its intrinsic resistance to many antibiotics make it a formidable pathogen in cancer patients, where immune suppression can lead to aggressive infections.

Serratia species, particularly *S.ficaria* and *S.odorifera*, represented 11.53% and 7.69% of the total isolates, respectively. Although these species are less frequently associated with human infections, their presence in breast and colorectal cancers suggests that they may act as emerging pathogens in these patient groups. The detection of *A.baumannii* exclusively in breast cancer cases further underscores the diversity of opportunistic infections encountered in cancer care.

Our analysis revealed a balanced distribution of bacterial isolates between males and females, with no significant association between gender and bacteria species. However, some species, such as *S.ficaria* and *S.odorifera*, were predominantly isolated from female patients, whereas *S.rubidaea*, *S.maltophilia*, and *C.indologenes* were detected exclusively in males. The absence of a significant gender correlation suggests that bacterial colonization and infection are likely to be influenced by cancer type and patient-specific factors rather than gender.

Our findings in this study demonstrate the high rate of antibiotic resistance among the isolated bacteria across all species. 100% resistance to amoxicillin/clavulanic acid (AMC30) and cefotaxime (CTX30), was observed particularly in species such as *S.odorifera*, *K.pneumoniae*, and *A.baumannii*. This widespread resistance highlights the growing challenge of managing bacterial infections in cancer patients, where limited antibiotic options remain effective.

The resistance profile of *E.coli* is particularly alarming, with 66.66% resistance to tetracycline (TE30), ciprofloxacin (CIP5), and chloramphenicol (CHL30). Given the high prevelence of *E. coli* in breast and colorectal cancer patients, these resistance patterns

suggest the need for targeted infection control measures and the judicious use of antibiotics in these populations. Similarly, the resistance of *P.aeruginosa* to multiple antibiotics, including penicillin, amikacin, and carbapenems, underscores the difficulty in treating infections caused by this pathogen [18].

The beta-lactams (penicillin group) exhibited an average resistance rate of 71.71%, further complicating the challenge of treating infections in cancer patients. Aminoglycosides, however, demonstrated a significantly lower resistance rate (14.89%), offering a potential alternative for treatment, particularly in cases involving *E.coli* and *K.pneumoniae*. On the other hand, the high resistance to beta-lactamase inhibitors and carbapenems suggests that infections caused by these bacteria may require more advanced or combination therapies [19].

The ability of several bacterial species to form biofilms, as demonstrated by the OD595 measurements, is another critical factor in their persistence and resistance. *E.coli*, *K.pneumoniae*, and *P.aeruginosa* exhibited strong biofilm formation capabilities, which may contribute to their resistance to antibiotics and ability to cause chronic infections in cancer patients[20].

Melatonin's potential as an anti-biofilm agent represents a promising approach to mitigating chronic bacterial infections in cancer patients, particularly those involving biofilm-forming solid species such as *E.coli*, *K.pneumoniae*, and *P.aeruginosa* [21]. The inhibition of *E.coli* biofilm formation with increasing melatonin concentrations suggests that melatonin may interfere with biofilm maturation or disrupt biofilm-related pathways[22]. At a concentration of 1000 µg/mL, *E.coli* biofilm formation was significantly reduced, highlighting the potential of melatonin to impair the biofilm structure and enhance bacterial susceptibility to antibiotics. This is especially relevant in cancer patients, where persistent biofilms can complicate infection management and reduce the efficacy of standard treatments.

The effects of melatonin on other important biofilm-forming bacteria, such as *K.pneumoniae* and *P.aeruginosa*, appeared to be slower and less significant. This suggests that the ability of melatonin to combat biofilms may differ between species. In particular, *K.pneumoniae* showed some resistance to melatonin at lower concentrations, maintaining its biofilm structure until higher doses were introduced. This suggested that for bacteria with strong biofilm-forming abilities, such as *K.pneumoniae*, melatonin alone may not be enough to break up biofilms. It may need to be used alongside traditional antibiotics for complete biofilm elimination [23 24].

The results with *P.aeruginosa*, known for its resistant biofilm formation and innate resistance mechanisms, further highlight this challenge. Despite some reduction in biofilm mass at higher melatonin concentrations, *P.aeruginosa* displayed consistent resistance to the effects of melatonin. This resistance may be due to the bacterium's ability to modify its biofilm architecture or upregulate efflux systems that mitigate the effects of melatonin[25]. Therefore, treating biofilm-associated infections caused by *P.aeruginosa* may require a multifaceted approach, combining melatonin with other antibiofilm or antimicrobial agents to enhance efficacy.

The antibiofilm activity of melatonin highlights the need for further research to understand the molecular mechanism by which melatonin excerts its effects on the biofilm formation mechanism. Further research is needed into how melatonin interacts with bacterial quorum sensing pathways, biofilm matrix production, and other regulatory systems which could provide valuable insights into optimizing its use in clinical settings. In addation, exploring synergistic combinations of melatonin with antibiotics may improve therapeutic outcomes, particularly against multi-drug resistant biofilm-associated infections in cancer patients[26 27].

CONCLUSION

This study highlights the complexity of bacterial infections in cancer patients, characterized by various pathogens with varying antibiotic resistance profiles. The high prevalence of multidrug resistant bacteria, such as *E.coli*, *K.pneumoniae*, and *P.aeruginosa*, highlights the need for improved infection control strategies and more judicious use of antibiotics in cancer care. Furthermore, the antibiofilm potential of melatonin offers new avenues for exploring alternative therapies to combat persistent bacterial infections. Future research should focus on expanding the sample size and investigating the underlying mechanisms driving the observed patterns of bacterial prevalence and resistance across different cancer types.

Declarations:

Ethical Approval:

Not applicable, because this article does not contain any human participants, data or tissues.

Consent for publication:

Not applicable

Availability of data and material:

Data are available bupon request.

Competing interests:

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REFERENCES

1. Tohamy ST, Aboshanab KM, El-Mahallawy HA, El-Ansary MR, Afifi SS. Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with bloodstream infections in Egypt and new synergistic antibiotic combinations. Infect Drug Resist. 2018;791–803.
2. Perdikouri EIA, Arvaniti K, Lathyris D, Apostolidou Kiouti F, Siskou E, Haidich AB, et al. Infections due to multidrug-resistant bacteria in oncological patients: insights from a five-year epidemiological and clinical analysis. Microorganisms. 2019;7(9):277.
3. Gopichand P, Agarwal G, Natarajan M, Mandal J, Deepanjali S, Parameswaran S, et al. In vitro effect of fosfomycin on multi-drug resistant gram-negative bacteria causing urinary tract infections. Infect Drug Resist. 2019;2005–13.
4. Fathy FEY, Abdel Salam SA, Ahmed YM. Antimicrobial profile of pathogens causing community acquired urinary tract infection in Ain Shams University hospitals. Egypt J Med Microbiol. 2023;32(2):17–24.
5. Abbas SM, Dhahi MAR. Evaluation of some virulence factors and drug resistance of bacteria isolated from the urine of patients with TCC-bladder cancer. Baghdad Science Journal. 2022;19(3):469.
6. Gudiol C, Carratalà J. Antibiotic resistance in cancer patients. Expert Rev Anti Infect Ther. 2014;12(8):1003–16.
7. Bonmati-Carrion MA, Tomas-Loba A. Melatonin and cancer: A polyhedral network where the source matters. Antioxidants. 2021;10(2):210.
8. He F, Wu X, Zhang Q, Li Y, Ye Y, Li P, et al. Bacteriostatic potential of melatonin: therapeutic standing and mechanistic insights. Front Immunol. 2021;12:683879.
9. Chakraborty S, Sarkar S, Chattopadhyay A, Bandyopadhyay D. The dual-actions of melatonin as a potential oncostatic agent and a protector against chemotherapy-induced toxicity. Melatonin Research. 2023;6(2):189–214.
10. Ghallab AF, Hashim HF, Mostafa MS, El Sayed RA. The Role of the Bacterial Infections of the Nose in Etiology of Primary Atrophic Rhinitis. Egypt J Med Microbiol. 2020;29(2):117–21.
11. Nakasone I, Kinjo T, Yamane N, Kisanuki K, Shiohira CM. Laboratory-based evaluation of the colorimetric VITEK-2 Compact system for species identification and of the Advanced Expert System for detection of antimicrobial resistances: VITEK-2 Compact system identification and antimicrobial susceptibility testing. Diagn Microbiol Infect Dis. 2007;58(2):191–8.
12. Hombach M, Bloemberg G V, Böttger EC. Effects of clinical breakpoint changes in CLSI guidelines 2010/2011 and EUCAST guidelines 2011 on antibiotic susceptibility test reporting of Gram-negative bacilli. Journal of antimicrobial chemotherapy. 2012;67(3):622–32.
13. Alsaadi AG, Shoukr TG, Talaat RZ. Microbiological Profile, Biofilm Formation and Antimicrobial Resistance Pattern of Pathogenic Bacteria Isolated from Chronic Ulcerative Lesions. Egypt J Med Microbiol. 2022;31(3):9–16.
14. Kang CI, Chung DR, Son JS, Ko KS, Peck KR, Song JH, et al. Clinical significance of nosocomial acquisition in urinary tract–related bacteremia caused by gram-negative bacilli. Am J Infect Control. 2011;39(2):135–40.
15. Olvera A, Carter H, Rajan A, Carlin LG, Yu X, Zeng XL, et al. Enteropathogenic Escherichia coli infection in cancer and immunosuppressed patients. Clinical Infectious Diseases. 2021;72(10):e620–9.
16. Tohamy ST, Aboshanab KM, El-Mahallawy HA, El-Ansary MR, Afifi SS. Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with bloodstream infections in Egypt and new synergistic antibiotic combinations. Infect Drug Resist. 2018;791–803.
17. Shahid M, Malik A, Akram M, Agrawal LM, Khan AU, Agrawal M. Prevalent phenotypes and antibiotic resistance in Escherichia coli and Klebsiella pneumoniae at an Indian tertiary care hospital: plasmid-mediated cefoxitin resistance. International journal of infectious diseases. 2008;12(3):256–64.
18. Sharma S, Magar PT, Achhami S, Hamal P, Chapagain B, Jaiswal S, et al. Bacterial profiling and antibiotic-resistant pattern among cancer patients. Clin Cancer Investig J. 2021;11(2):14–20.
19. Rashed HG, Ahmed AO, Moawad AK. Different Phenotypic Methods for Detection of Beta Lactam Resistance in Escherichia coli. Egypt J Med Microbiol. 2019;28(2):19–26.
20. Reffuveille F, De La Fuente-Nűnez C, Mansour S, Hancock REW. A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. Antimicrob Agents Chemother. 2014;58(9):5363–71.
21. Liu Y, Jia Y, Yang K, Tong Z, Shi J, Li R, et al. Melatonin overcomes MCR-mediated colistin resistance in Gram-negative pathogens. Theranostics. 2020;10(23):10697.

22. Chen X, Sun C, Laborda P, Zhao Y, Palmer I, Fu ZQ, et al. Melatonin treatment inhibits the growth of Xanthomonas oryzae pv. oryzae. Front Microbiol. 2018;9:2280.
23. Sajna KP, Paulraj P, Sajeesh P, Jayakumar N. Role of multivitamins on the development of antibiotic resistance associated with biofilm formation on central venous catheters—An in vitro study. Mater Today Proc. 2019;16:1581–1589.
24. Graniczkowska KB, Shaffer CL, Cassone VM. Transcriptional effects of melatonin on the gut commensal bacterium Klebsiella aerogenes. Genomics. 2022;114:110321.
25. Akinduti PA, George OW, Ohore HU, Ariyo OE, Popoola ST, Adeleye AI, et al. Evaluation of efflux-mediated resistance and biofilm formation in virulent Pseudomonas aeruginosa associated with healthcare infections. Antibiotics. 2023;12(3):626.
26. Ganganna A, Rudariah CB, Rao R, Prakash VM. Antibacterial activity of melatonin against prime periodontal pathogens: An in vitro study. J Int Oral Health. 2021;13(2):164–8.
27. Mohamed Y, Basyony MA, El-Desouki NI, Abdo WS, El-Magd MA. The potential therapeutic effect for melatonin and mesenchymal stem cells on hepatocellular carcinoma. BioMedicine. 2019;9(4):23–29.