Journal of Bioresource Management

Volume 9 | Issue 1 Article 12

Current Epidemiological Status and Antibiotic Resistance Profile of Urinary Tract Infection

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Recommended Citation

Khan, M., Shah, S. H., Abdullah, M., Salman, M., Hayat, F., Sarwar, R., Hassan, N., Jan, S. A., Saddam, M., Rahman, Z., & Qazi, I. (2022). Current Epidemiological Status and Antibiotic Resistance Profile of Urinary Tract Infection, *Journal of Bioresource Management, 9* (1).
ISSN: 2309-3854 online
(Received: Nov 9, 2021; Accepted: Jan 14, 2022; Published: Mar 24, 2022)

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Cover Page Footnote

The authors are thankful to all staff of the Complex medical laboratory for providing the platform and support.

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CURRENT EPIDEMIOLOGICAL STATUS AND ANTIBIOTIC RESISTANCE PROFILE OF URINARY TRACT INFECTION

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ABSTRACT

With a longitudinal (2020–2021) completion, a set of 300 urine samples of individuals suspected of UTI was characterized based on the biochemical analysis and Kirby Bauer's disc diffusion method. Throughout, a total of 167 samples revealed UTI positivity by obtaining bacterial growth. Reporting that *E. coli* (69.4 %) was the most dominant when compared with *Klebsiella* (14.3 %), *Proteus* (5.9 %), *S. aureus* (4.7 %), *P. aeruginosa* (3.5 %), and *Enterobacter* (1.7 %), respectively. Gender-wise discrimination showed that *E. coli* was highly distributed among female (50.2 %) patients. A prominent percentage of *E. coli* (41.9 %) was found in the age group between 21-40 years. Isolates of *E. coli* represented the highest degree of resistance to Amoxicillin and Ceftriaxone (85.3 %), while highly sensitive to Amikacin, and Tazobactam (94.8 %). *S. aureus* was found highly resistant to Amoxicillin, Levofloxacin, Sulbactam, and Doxycycline (75 %), while highly sensitive to Meropenem, and Cefotaxime (100 %). *Proteus* spp. was found highly resistant to Sulbactam, and Cefotaxime (100 %), while highly sensitive to Tazobactam (100 %). *Enterobacter* was found highly resistant to Gentamicin (100 %), while highly sensitive to Aztreonam (100 %). *Klebsiella* was found highly resistant to Levofloxacin, and Doxycycline (91.6 %), while highly sensitive to Amikacin, Tazobactam, Gentamicin, and Cefoxitin (75 %). *P. aeruginosa* was found highly resistant to Nalidixic acid (100 %), while highly sensitive to Meropenem, Ceftriaxone, Gentamicin, and Cefotaxime. In conclusion, the Amikacin and Tazobactam were seen to be effective for empirical therapy of UTI.

Keywords: Epidemiology, antibiotic sensitivity pattern, uropathogens, Peshawar.

INTRODUCTION

Urinary tract infection (UTI) is one of the most significant bacterial infections in the 21st century causing various types of health concerns in both males and females. Although both genders are exposed to the infection, women are more susceptible due to their reproductive physiology and morphology as compared to males (Vasudevan, 2015). UTI is one of the most widespread infectious diseases found in outpatients, and could also be caused by certain fungi and viruses as well as bacteria (Flores-Mireles et al., 2015). According to the authors, more than 80 % of UTI is caused by Gram-negative bacteria and other due to Gram-positive bacteria. Many factors are associated with the prevalence of UTI particularly the presence of more than 105 CFU/ml bacteria in the urine. *Escherichia coli* is the most prevalent uropathogen *(*60-90 %), followed by *Staphylococcus* species accounts for 10-15 % UTIs.

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In addition, other bacterial species such as *Streptococcus*, *Proteus*, *Klebsiella*, *Neisseria*, *Corynebacterium*, and *Pseudomonas* are also isolated from patients with UTI also reported (Ashur et al., 2021; Seifu and Gebissa, 2018). UTI is symptomatic sometimes but typically the clinical symptoms of UTI include urgency, painful urination, feelings of urination after urination, dysuria, pyuria, pain in the back, and abdominal pain. Other host-related factors increase the rate of urinary tract infection, including; urine factors, sexual factors, osmolality, vaginal pH, and secretory condition (Nigussie and Amsalu, 2017).

The treatment of UTI is a big hurdle for physicians. The emergence of antibiotic resistance among uropathogens has increased globally and become a serious public health issue especially in low-income countries (Ejerssa et al., 2021). According to a recent study in Europe, UTI treatment accounts for 9 % of all antibiotic prescriptions, and patients with UTIs showed resistance to antibiotics in a high ratio. Drug-resistant bacterial strains, as well as the high prevalence of UTI, underline the need for a better understanding of the microorganisms that cause UTI and their pattern of antibiotic resistance (Folliero et al., 2020). The severity of a UTI is dictated by the virulence of the bacteria as well as the host's susceptibility (Hannan et al., 2012). Because of antibiotic overuse, uropathogen isolates now have an unacceptably high proportion of resistance to practically all antibiotics across the world (Khan et al., 2021; Gebremariam et al., 2019).

In context to an abovementioned health issue, the current study was designed to investigate the epidemiological status of uropathogens, as well as their pattern of resistance to commonly used antibiotics, in physician-referred UTI patients in Peshawar, Pakistan.

MATERIALS AND METHODS

Study Design

A cross-sectional study was conducted from February 2020 to January 2021 at Complex medical laboratory and Diagnostic Center, Peshawar, Pakistan.

Samples Collection and Handling

Total 300 urine samples from UTI suspect patients referred by physicians were undertaken at the Complex medical laboratory and Diagnostic Center in Peshawar, Pakistan. Clean capture mid-stream urine samples were obtained and labeled from all UTI suspicion patients. The patients' names, ages, genders, clinical histories, and treatment histories were recorded. The following four age groups were included in this study ≤20, 21-40, 41-60, and ≥61 years.

Initial Examination of Urine samples

Initially, urine samples were screened for nitrite, protein, glucose, ketone bodies, bilirubin, bile pigments, red blood cells, and pus cells by visual, dipstick, and microscopic examination. Urine culture samples had more than four leukocytes per field, more than two red blood cells per field, numerous epithelial cells, and crystals (Cristina et al., 2009).

Isolation of Bacteria

Urine samples were streaked on Blood agar medium and MacConkey Agar media with a 0.01 mL calibrated wire loop. For 24 hours, at 37^{o}C aerobically, all plates were incubated. Positive samples showed growth, and isolated colonies were Gram stained. The pure isolated colony culture was then processed for biochemical examination to identify pathogens. To identify uropathogens, the following tests were performed: Oxidase test, Catalase test, Indole test Urease test, Citrate, Utilization test, Methyl red test, H2S,

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Voges Proskauer test, Gas output test, and Motility test (Khan et al., 2021).

Antibiotic Susceptibility Testing

The concentration of antibiotics discs (Oxoid, Ltd, England) used in the Kirby Bauer's disc diffusion test and Clinical and Laboratory Standards Institute (CLSI), 2019 standards was Amoxicillin (AMC) 30 µg, Amikacin (AK) 30 µg, Levofloxacin (LEV) 5 µg, Tazobactam (TZP) 110 µg, Meropenem (MEM) 10 µg, Sulbactam (SAM) 20 µg, Ceftriaxone (CRO) 30 µg, Ciprofloxacin (CIP) 5 µg, Cefotaxime (CTX) 30 µg, Doxycycline (DXT) 30 µg, Aztreonam (ATM) 30 µg, Nalidixic acid (NA) 30 µg, Gentamicin (CN) 10 µg, and Cefoxitin (FOX) 30 µg. The inoculum was prepared for 2 mL of Muller Hinton broth and cultured for 4 hours at 37^{o}C to generate fresh culture and then applied to the McFarland 0.5 standard. The Muller Hinton agar plate surface was stripped with a sterile cotton swab that had been submerged in the solution. Aseptically, antibiotic discs were placed with sterile forceps on the agar surface and for 24 hours the plates were incubated at 37^{o}C. After incubation, millimeter calipers were used to measure the widths of the zones of complete inhibition (Khan et al., 2021).

Quality Control

Reference strain stranded for quality control for culture and sensitivity checking, *E. coli* (ATCC 25922) was used. The zone of isolate inhibition was graded as susceptible and resistant according to CLSI criteria (29th Edition CLSI, 2019).

ETHICAL STATEMENT

The Complex Medical Laboratory and Diagnostic Center in Peshawar and Abasyn University in Peshawar, Pakistan, ethical committees approved the study.

RESULTS

A total of 300 urine samples were investigated for UTI. Among these, 167 (55.6 %) were positive for bacterial growth in both genders (20-61 years age**).** The most frequent bacterial isolates were *E. coli* n=116 (69.4 %), followed by *Klebsiella* spp. n=24 (14.3 %), *Proteus* spp. n=10 (5.9 %), *S. aureus* n=8 (4.7 %), *P. aeruginosa*, n=6 (3.5 %) and *Enterobacter* n=3 (1.7 %) respectively (Fig. 1). The current study reported high frequency of *E. coli* in female(s) n=84 (50.2 %) as compared with n=32 (19.1 %). The highest frequency of Gram-negative isolates *E. coli* n=70 (41.9 %) age group range from 21-40 years, followed by vulnerable 41-60 years with n=20 (11.9 %), n=14 (8.3 %) ≥61 years, n=12 (7.1 %) (Table 1).

It was observed that *E. coli* strains displayed relatively highest degree of resistance to Amoxicillin and Ceftriaxone (85.3 %), while highly sensitive to Amikacin, and Tazobactam (94.8 %). *S. aureus* was found highly resistant to Amoxicillin, Levofloxacin, Sulbactam, and Doxycycline (75 %), while highly sensitive to Meropenem, and Cefotaxime (100 %). *Proteus* spp. was found highly resistant to Sulbactam, and Cefotaxime (100 %), while highly sensitive to Tazobactam (100 %). *Enterobacter* was found highly resistant to Gentamicin (100 %), while highly sensitive to Aztreonam (100 %). *Klebsiella* spp. was found highly resistant to Levofloxacin, and Doxycycline (91.6 %), while highly sensitive to Amikacin, Tazobactam, Gentamicin, and Cefoxitin (75 %). *P. aeruginosa* was found highly resistant to Nalidixic acid (100 %), while highly sensitive to Meropenem, Ceftriaxone, Gentamicin, and Cefotaxime (100 %) (Table 2).

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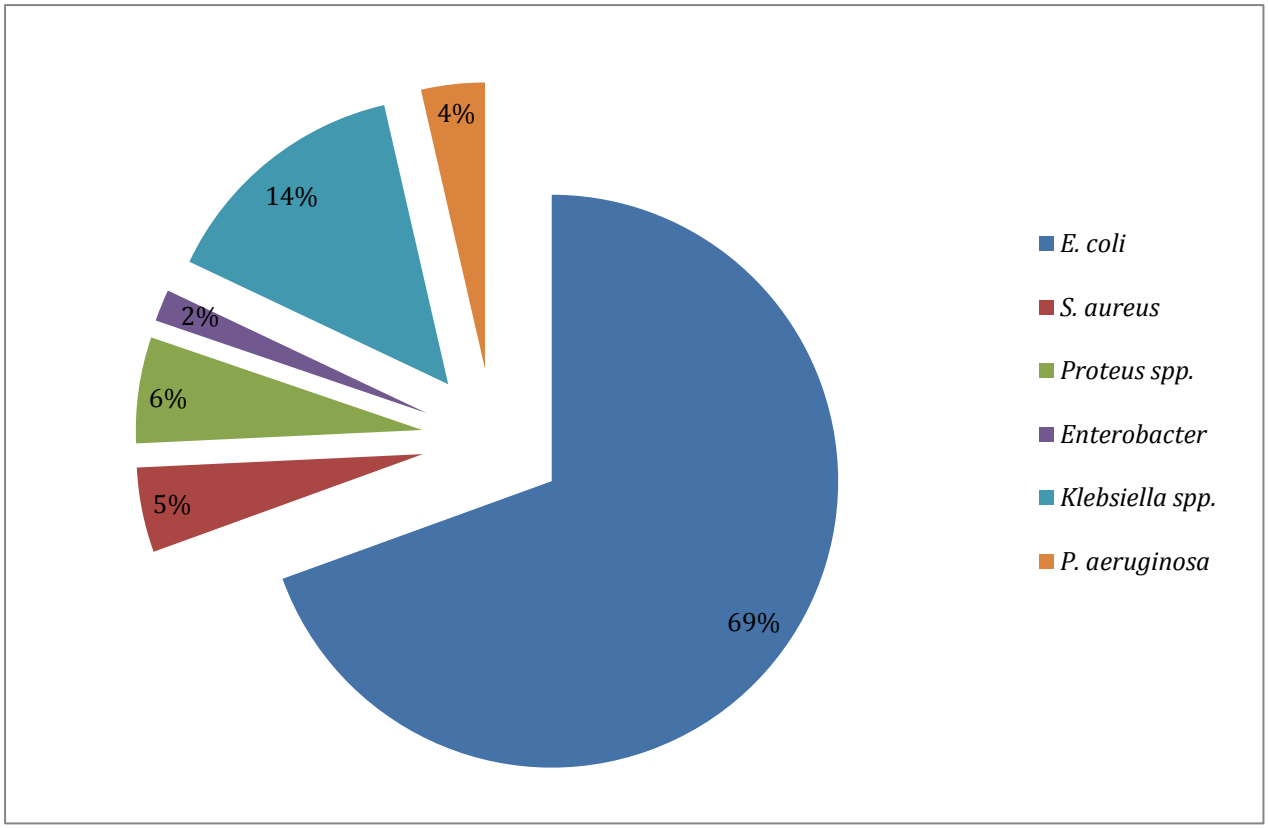

Figure 1: Overall distribution of Uropathogens.

Table 1: Overall, gender wise and age wise distribution of Uropathogens.

Overall Etiology of Uropathogens						
Total = 167	***E. coli* n (%)**	***S. aureus* n (%)**	***Proteus* spp. n (%)**	***Enterobacter* n (%)**	***Klebsiella* spp. n (%)**	***P. aeruginosa* n (%)**
	116 (69.4 %)	8 (4.7 %)	10 (5.9 %)	3 (1.7 %)	24 (14.3 %)	6 (3.5 %)
Gender Wise Etiology of Uropathogens						
Male	32 (19.1 %)	6 (3.5 %)	8 (4.7 %)	1 (0.5 %)	10 (5.9 %)	5 (2.9 %)
Female	84 (50.2 %)	2 (1.1 %)	2 (1.1 %)	2 (1.1 %)	14 (8.3 %)	1 (0.5 %)
Age Wise Etiology of Uropathogens						
≤20	12 (7.1 %)	1 (0.5 %)	1 (0.5 %)	0 (0 %)	4 (2.3 %)	1 (0.5 %)
21-40	70 (41.9 %)	3 (1.7 %)	5 (2.9 %)	2 (1.1 %)	11 (6.5 %)	3 (1.7 %)
41-60	20 (11.9 %)	3 (1.7 %)	3 (1.7 %)	1 (0.5 %)	6 (3.5 %)	2 (1.1 %)
≥61	14 (8.3 %)	1 (0.5 %)	1 (0.5 %)	0 (0 %)	3 (1.7 %)	0 (0 %)

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Table 2: Antibiotics sensitivity and resistance pattern of Uropathogens.

Antibiotics Disc	*E. coli* (n=116)		*S. aureus* (n=08)		*Proteus* spp. (n=10)		*Enterobacter* (n=03)		*Klebsiella* spp. (n=24)		*P. aeruginosa* (n=06)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Amikacin (AK) 30µg	110 (94.8 %)	6 (5.1 %)	4 (50 %)	4(50 %)	8 (80 %)	2 (20 %)	3 (100 %)	0 (0 %)	18 (75 %)	6 (25 %)	5(83.3 %)	1(16.6 %)
Amoxicillin (AMC) 30µg	17 (14.6 %)	99 (85.3 %)	2 (25 %)	6 (75 %)	9 (90 %)	1 (10 %)	2 (66.6 %)	1 (33.3 %)	6 (25 %)	18 (75 %)	3(50 %)	3(50 %)
Levofloxacin (LEV) 5µg	57 (49.1 %)	59 (50.8 %)	2 (25 %)	6 (75 %)	2 (20 %)	8 (80 %)	3 (100 %)	0 (0 %)	2 (8.3 %)	22 (91.6 %)	4(66.6 %)	2(33.3 %)
Ciprofloxacin (CIP) 5µg	40 (34.4 %)	76 (65.5 %)	3 (37.5 %)	5 (62.5 %)	5 (50 %)	5 (50 %)	3 (100 %)	0 (0 %)	10 (41.6 %)	14 (58.3 %)	4(66.6 %)	2(33.3 %)
Tazobactam (TZP) 110µg	110 (94.8 %)	6 (5.1 %)	7 (87.5 %)	1 (12.5 %)	10 (100 %)	0 (0 %)	1 (33.3 %)	2 (66.6 %)	18 (75 %)	6 (25 %)	3(50 %)	3(50 %)
Meropenem (MEM) 10µg	87 (75 %)	29 (25 %)	8 (100 %)	0 (0 %)	9 (90 %)	1 (10 %)	3 (100 %)	0 (0 %)	12 (50 %)	12 (50 %)	6(100 %)	0(0 %)
Sulbactam (SAM) 20µg	29 (25 %)	87 (75 %)	2 (25 %)	6 (75 %)	0 (0 %)	10 (100 %)	3 (100 %)	0 (0 %)	4 (16.6 %)	20 (83.3 %)	4(66.6 %)	2(33.3 %)
Ceftriaxone (CRO) 30µg	17 (14.6 %)	99 (85.3 %)	7 (87.5 %)	1 (12.5 %)	2 (20 %)	8 (80 %)	1 (33.3 %)	2 (66.6 %)	10 (41.6 %)	14 (58.3 %)	6(100 %)	0(0 %)
Gentamicin (CN) 10µg	58 (50 %)	58 (50 %)	7 (87.5 %)	1 (12.5 %)	8 (80 %)	2 (20 %)	0 (0 %)	3 (100 %)	18 (75 %)	6 (25 %)	6(100 %)	0(0 %)
Cefotaxime (CTX) 30µg	29 (25 %)	87 (75 %)	8 (100 %)	0 (0 %)	0 (0 %)	10 (100 %)	3 (100 %)	0 (0 %)	6 (25 %)	18 (75 %)	6(100 %)	0(0 %)
Doxycycline (DXT) 30µg	45 (38.7 %)	71 (61.2 %)	2 (25 %)	6 (75 %)	9 (90 %)	1 (10 %)	2 (66.6 %)	1 (33.3 %)	2 (8.3 %)	22 (91.6 %)	1(16.6 %)	5(83.3 %)
Aztreonam (ATM) 30µg	23 (19.8 %)	93 (80.1 %)	4 (50 %)	4(50 %)	2 (20 %)	8 (80 %)	0 (0 %)	3 (100 %)	12 (50 %)	12 (50 %)	1(16.6 %)	5(83.3 %)
Nalidixic acid (NA) 30µg	23 (19.8 %)	93 (80.1 %)	3 (37.5 %)	5 (62.5 %)	9 (90 %)	1 (10 %)	2 (66.6 %)	1 (33.3 %)	12 (50 %)	12 (50 %)	0(0 %)	6(100 %)
Cefoxitin (FOX) 30µg	87 (75 %)	29 (25 %)	7 (87.5 %)	1 (12.5 %)	8 (80 %)	2 (20 %)	1 (33.3 %)	2 (66.6 %)	18 (75 %)	6 (25 %)	1(16.6 %)	5(83.3 %)

DISCUSSION

The over or misuse of antibiotics to treat infections has deleterious consequences on public health as well as socio-economic impact and that increase resistance among causative agents such as bacteria. In context to this, it is critical to assess the status of antimicrobial resistance in the community. The main objective of this study is to achieve this goal in UTI patients. In this study prevalence of UTI in referred outpatients was 55.6 %, the study has similar findings (80 %) with Sangsuwan et al., (2021) study conducted in Thailand (Sangsuwan et al.,

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2021). The current study investigated 300 urine samples 167 study samples *E. coli* were found in (69.4 %) patients, *E. coli* were high in female (50.2 %) patients, while in males were (19.1 %). In different studies conducted, similarly elevated prevalence rates of UTI incidence in women were identified include Turkey (82.30 %), Iran (86.24 %), Saudi Arab (60.35 %), Pakistan (69.80 %), and Italy (60 %) (Sabir et al., 2014; Khan et al., 2014; Al-Mijalli et al., 2017; Farajnia, 2009; Folliero et al., 2020). The present study also revealed the age group 21-40 years as the most susceptible group to *E. coli* (69.4 %) among the uropathogens. Our findings based on age group are similar to the study conducted by Shabir et al. 2017 in Peshawar, Pakistan. The authors revealed patients with age group susceptibility to *E. coli* that can be ordered from 41 to 60 years of age (11.9 %), from ≥60 years of age (8.3 %), and from ≤20 years of age (7.1 %). The current study is inconsistent with a previously conducted study where 65 % of patients with age >60 years were more exposed to infection (Sangsuwan et al., 2021). The aforementioned variation in incidence could be explained by geography differences, and other associated factors such as social habits, personal hygiene, and health education awareness and practices. Inlined with higher maximum UTI cases in women certain factors may be associated including the small urethra, deficiency of germicidal substances in prostatic fluid, hormonal imbalance (Akter et al., 2016). The current study observed *E. coli as* the most frequent uropathogen *(69 %)* in UTI. Our findings in this context are in accordance with previously conducted studies in Pakistan and also other parts of the world (Seifu and Gebissa, 2018; Folliero et al., 2020; Qazi & Ainuddin, 2021; Shabir et al., 2017). The current study also observed that *E. coli* is sensitive to Amikacin, and Tazobactam (94.8 %). This study is consistent with previous studies where the sensitivity rate to Amikacin was (92.5 %) and to Tazobactam was (96.2 %) (Ho et al., 2019; Sangsuwan et al., 2021; Shabir et al., 2017; Hussain et al., 2021**).**

In addition, high levels of resistance were noted to Ceftriaxone and Amoxicillin were (85.3 %); Aztreonam and Nalidixic Acid were (80.1 %). This finding of high Ceftriaxone resistance correlates with the previous study conducted by Shabir et al., 2017 in Peshawar, Pakistan, where the resistance rate to Ceftriaxone was (78.0 %). Increasing resistance (over 60 %) of the UTI strain to Nalidixic Acid was also seen in a study in Pakistan (Liaqat et al., 2014). Similar studies in Iraq, on the other hand, found a lower resistance rate (25.9 %) of the *E. coli* strain to Nalidixic Acid (Aaboda and Notazy, 2018). Resistance to Nalidixic Acid is suspected to have arisen because of the overabundant use of first-generation quinolone Nalidixic Acid for the treatment of UTI (Akter et al., 2016; Johnson et al., 2003).

CONCLUSION

The present study concluded that *E. coli* is the most prevalent uropathogens followed by *Klebsiella* spp., *Proteus* spp., *S. aureus*, *P. aeruginosa*, and *Enterobacter* in the study area. The highest percentage uropathogens were found in females and 21-40 age groups. Amikacin and Tazobactam were found effective for empirical therapy of UTI from the antimicrobial susceptibility profile. It is also recommended that doctors examine the Antibiotic resistance to a patient's prescription UTI pathogen. Usage of antibiotics to combat infections and their resistance is important to track and regulate. Future studies are suggested for the detection of other UTI isolates and the use of all antibiotics in assessing elaborate susceptibility trends.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. All authors read and approved the final version.

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