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Original article

Molecular Characterization of Sulfonamide and Fluoroquinolone Resistant Genes of *Morganella morganii* Isolates from Urinary Tract Infections (UTIs) Patients, Peshawar

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Abstract: Uropathogens pose a significant threat to public health, affecting both community and hospitalized patients. Among these, Gram-negative bacteria represent a predominant group. In recent years, *Morganella morganii* has emerged as a notable cause of urinary tract infections (UTIs) worldwide, with a concerning rise in prevalence attributed to the evolution of resistance mechanisms against multiple antibiotics. The present study aimed to investigate the prevalence of M. morganii, its associated antibiotic-resistance genes, and its correlation with demographic factors such as gender and age. The findings revealed that M. morganii was more frequently isolated from females (n = 27) than males (n = 16). The highest prevalence rate (37.2%) was observed in the age group of 21–40 years. The antibiotic susceptibility profile of M. morganii isolates indicated high resistance to cotrimoxazole (77%), followed by ciprofloxacin (67%), Cefoperazone (65%), cefotaxime (58%), gentamicin (28%), amoxicillin-clavulanate (26%), imipenem (16%), Fosfomycin (9%), meropenem (7%), piperacillin-tazobactam (7%), and amikacin (5%). PCR analysis revealed the presence of antibiotic-resistance genes in the resistant isolates. Among cotrimoxazole-resistant isolates, the sul1 and sul2 genes were detected in 23 isolates each. Similarly, in ciprofloxacin-resistant isolates, the *gyrA* and *gyrB* genes were identified in 11 and 10 isolates, respectively. Interestingly, certain isolates harbored various combinations of these resistance genes. Seven isolates contained all four resistance genes (*sul1*, *sul2*, *gyrA*, and *gyrB*), while seven isolates harbored three genes in combinations (*sul1*, *sul2*, *gyrA* or *sul1*, *sul2*, *gyrB*). Nine isolates exhibited two-gene combinations (*sul1* and *sul2*).

Keywords: uropathogen, urinary tract infections (UTIs), sul1, sul2, gyrA, gyrB, PCR analysis.

1. Introduction

Globally, the prevalence of urinary tract infections (UTIs) remains significantly high, largely due to the emergence of multidrug-resistant bacterial strains. UTIs include urethritis (infection of the urethra), cystitis (infection of the bladder), and pyelonephritis (infection of the kidney). While UTIs affect individuals of all genders and age groups, females are more susceptible due to their physiological urethral structure. Approximately 60% of females experience at least one episode of UTI in their lifetime. The primary causative agents of UTIs are members of the *Enterobacteriaceae* family, responsible for approximately 80% of UTI cases [1]. *Morganella morganii*, a commensal organism of the human

intestine, has emerged as a significant uropathogen due to its development of diverse multidrug resistance mechanisms [2]. *M. morganii* is a facultative anaerobic, Gram-negative bacillus found in the environment and as part of the normal microbiota of the human intestine. Under favorable conditions, it becomes opportunistic, causing severe clinical manifestations in vertebrates, reptiles, and humans. It is intrinsically resistant to several antibiotics, including ampicillin, oxacillin, amoxicillin, first- and second-generation cephalosporins, glycopeptides, colistin, Fosfomycin, and Fusidic acid. This intrinsic resistance makes the treatment of infections caused by *M. morganii* particularly challenging [3]. Morganella morganii is recognized as a significant pathogen responsible for both community- and hospital-acquired urinary tract infections (UTIs) [4]. Beyond UTIs, M. morganii has been implicated in a variety of severe infections in humans, including peritonitis [5][6], purple urine bag syndrome (PUBS) [7], pneumonia [8], arthritis [9], skin infections [10], neonatal meningitis [11], and sepsis [12]. Secondary infections caused by M. morganii are particularly prevalent in patients with diabetes mellitus, likely due to their compromised immune systems, which render them more susceptible to opportunistic infections [13]. The global rise in antimicrobial resistance (AMR) has exacerbated the challenges posed by bacterial infections. In 2016, AMR was attributed to approximately 700,000 deaths worldwide, and projections estimate that by 2050, it could lead to 10 million deaths annually if current trends continue [14]. The mechanisms by which bacteria develop resistance to antibiotics are diverse, but four primary pathways are frequently overexpressed and are central to AMR [15]. One major mechanism is the production of antibiotic-inactivating enzymes. These enzymes neutralize antibiotics, rendering them ineffective against the bacteria. Two prominent families of such enzymes include β-lactamases and aminoglycoside-modifying enzymes. β-lactamases, such as extended-spectrum β-lactamases (ESBLs), Carbapenemase, New Delhi metallo-β-lactamase (NDM-1), imipenem's (IMP), and Verona integrons-mediated metallo-β-lactamase (VIM), play a critical role in resistance to β-lactam antibiotics [16]. Similarly, aminoglycoside-modifying enzymes, including aminoglycoside phosphotransferases, aminoglycoside nucleotidyl transferases, and aminoglycoside acetyltransferases, confer resistance to aminoglycosides [17]. Another significant mechanism of resistance involves the modification of the antibiotic target site. This allows bacteria to alter the site of action, effectively preventing the antibiotic from binding and exerting its effect. For instance, β-lactam resistance is often associated with alterations in cell wall components, which reduce the antibiotic's ability to target the bacterial peptidoglycan layer [18]. Efflux pumps represent another critical resistance mechanism. These transmembrane proteins actively expel antibiotics from the bacterial cell, reducing their intracellular concentration and thereby limiting their efficacy. In addition, bacteria often employ modifications to their outer membrane to decrease its permeability, further restricting antibiotic entry into the cell and enhancing resistance. The combination of these adaptive resistance strategies underscores the formidable challenge posed by multidrug-resistant organisms such as *M. morganii*. Understanding these mechanisms is essential for developing innovative therapeutic approaches to mitigate the growing threat of AMR and improve patient outcomes. Fluoroquinolone resistance in bacteria is primarily associated with modifications in topoisomerase IV and DNA gyrase, mediated by mutations in the gyrA and gyrB genes [19]. Similarly, resistance to trimethoprim-sulfamethoxazole is commonly linked to mutations in the sul1, and sul2 genes [20]. An intrinsic feature of bacteria contributing to antibiotic resistance is the overexpression of efflux pump systems. These systems actively expel antibiotics from bacterial cells, thereby reducing their efficacy. Various efflux pump families have been identified in both Gram-positive and Gram-negative bacteria, including the Resistance Nodulation Division (RND) family, Small Multidrug Resistance (SMR) family, ATP-binding cassette (ABC) transporters, Multidrug and Toxin Extrusion (MATE) proteins, and the Major Facilitator Superfamily (MFS) [21]. Another

crucial mechanism of resistance is the low permeability of the bacterial outer membrane, which restricts the entry of hydrophilic antibiotics. This is often achieved through mutations in porins, the proteins responsible for forming channels in the outer membrane [22]. In recent years, *Morganella morganii* has emerged as a prevalent uropathogen with a well-developed arsenal of resistance mechanisms against antimicrobial agents. As such, determining the antibiotic susceptibility patterns of *M. morganii* is crucial for devising effective treatment strategies and managing infections caused by this pathogen. In the present study, the prevalence and antibiotic resistance patterns of *M. morganii* were investigated, with a focus on resistance to major antibiotic families. The study specifically examined the roles of fluoroquinolone resistance genes (*gyr*A and *gyr*B) and sulfonamide resistance genes (*sul1* and *sul2*) in *M. morganii* isolates. This research provides valuable insights into the mechanisms underlying resistance in *M. morganii* and emphasizes the importance of targeted antimicrobial therapies for managing infections caused by this pathogen.

2. Methodology

In the present study, urine samples from patients diagnosed with urinary tract infections (UTIs) were collected from the Outpatient Department (OPD) at Khyber Teaching Hospital, Peshawar, Pakistan. Further analyses were conducted at the Microbiology Laboratory, Centre of Biotechnology and Microbiology (COBAM), University of Peshawar. A total of 157 infected urine samples were collected. The samples were cultured on Cysteine Lysine Electrolytes Deficient (CLED) medium and MacConkey agar under optimal conditions (37°C for 24 hours). Following the incubation period, bacterial isolates were identified microscopically using Gram staining techniques and biochemically using the Analytical Profile Index (API 10S) kit [23]. The antibiotic susceptibility patterns of *Morganella morganii* clinical isolates were determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA). The isolates were cultured on MHA plates, and antibiotic discs were placed equidistantly on the agar surface. After 24 hours of incubation at 37°C, the zones of inhibition surrounding the discs were examined. The diameters of the zones of inhibition were measured and interpreted as sensitive, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) 2021 guidelines [24]. This methodological approach provided a comprehensive analysis of the resistance patterns of *M. morganii* isolates, aiding in the evaluation of its antimicrobial susceptibility profile.

Table 1: Antibiotics used for the determination of antibiogram of *Morganella morganii*

	Antibiotic	Symbol	Mode of action	Family
1	Ciprofloxacin	CIP	DNA gyrase	Fluoroquinolone
2	Cotrimoxazole	SXT	Folic acid metabolism	Sulfonamide
3	Fosfomycin	FOS	Cell wall synthesis	Phosphonic group
4	Amoxicillin-clavulanate	AMC	Cell wall synthesis	β-lactam (penicillin)
5	Piperacillin-tazobactam	TZP	Cell wall synthesis	β-lactam (penicillin)
6	Cefoperazone	CEP	Cell wall synthesis	β-lactam (cephalosporin)
7	Cefotaxime	CTX	Cell wall synthesis	cephalosporin)
8	Meropenem	MEM	Cell wall synthesis	β-lactam (carbapenem)
9	Imipenem	IPM	Cell wall synthesis	β-lactam (carbapenem)
10	Amikacin	AK	Protein synthesis, 30S subunit	Aminoglycoside
11	Gentamicin	CN	Protein synthesis, 30S subunit	Aminoglycoside

The genomic DNA of Morganella morganii isolates was extracted to detect antibiotic resistance genes using the GJC® DNA Purification Kit. DNA extraction was performed from 24-hour-old broth cultures of the isolates. The extracted DNA samples were subjected to gel electrophoresis on a 1.5% agarose gel and analyzed using a gel documentation system (Bio-Rad) [25]. Molecular detection of antibiotic resistance genes was carried out using a thermal cycler. The amplification of sulfonamide resistance genes (sul1 and sul2) and fluoroquinolone resistance genes (gyrA and gyrB) were performed under optimized conditions, as outlined in **Table 2**. Gene-specific primers were designed using the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The PCR reaction mixture included 12.5 µL of GoTaq® Green Master Mix, 1 µL of the upstream primer, 1 µL of the downstream primer, 11.5 µL of PCR-grade water, and 1 µL of target DNA. PCR amplification was carried out for 35 cycles under the specified conditions. The amplified products were then subjected to gel electrophoresis at 110 volts for 35 minutes on a 1.5% agarose gel stained with ethidium bromide. Visualization of the amplified bands was performed using a gel documentation system (Bio-Rad), and band sizes were estimated using a 100 bp DNA ladder (Promega BenchTop DNA Ladder) [26]. This approach enabled the precise identification of antibiotic-resistance genes in *M. morganii* isolates.

Table 2: Oligonucleotide sequences used for antibiotics resistance genes of M. morganii

Genes	Oligo-nucleotide sequences	Product size (bp)	Annealing Temperature
sul1	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	822bp	58˚C for 30 sec
sul2	F: CCTGTTTCGTCCGACACAGA R: GAAGCGCAGCCGCAATTCAT	435bp	58˚C for 30 sec
gyrA	F: ATGAGCGACCTTGCGAGAG R: TCCACTTCCGGAGCGATTTC	2600bp	59˚C for 30 sec
gyrB	F: GTAAGCGCCCGGGTATGTAT R: TCGATATTCGCCGCTTTCAG	2350bp	52˚C for 30 sec

After the detection of antibiotic-resistance genes, PCR products were subjected to mutational analysis. PCR products along with their primer working solution were sent to the Alpha Genomics Company. PCR products were sequenced through Next generation sequencing (NGS) method. Sequences of genes were properly aligned through BioEdit software (7.2) (https://bioedit.software.informer.com/7.2/). Consequence sequence of genes uploaded on NCBI blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) [26].

3. Results

A total of 157 clinical urine samples were collected from UTI patients. Among these, 43 (27.30%) isolates were confirmed as positive for the growth of Morganella morganii. The clinical isolates of M. morganii were identified microscopically and biochemically using the API 10S kit. The prevalence of *M. morganii* causing UTIs was higher in females (27 isolates, 63%) compared to males (16 isolates, 37%). The distribution of *M. morganii* prevalence was also assessed across different age groups of UTI patients. The highest prevalence rate (37.2%) was observed in the 21–40 years age group, followed by 32.5% in the 1–20 years age group, 14% in the 61–80 years age group, 11.6% in the 41–60 years age group, and 4.7% in infants under one year of age (**Figure 1**).

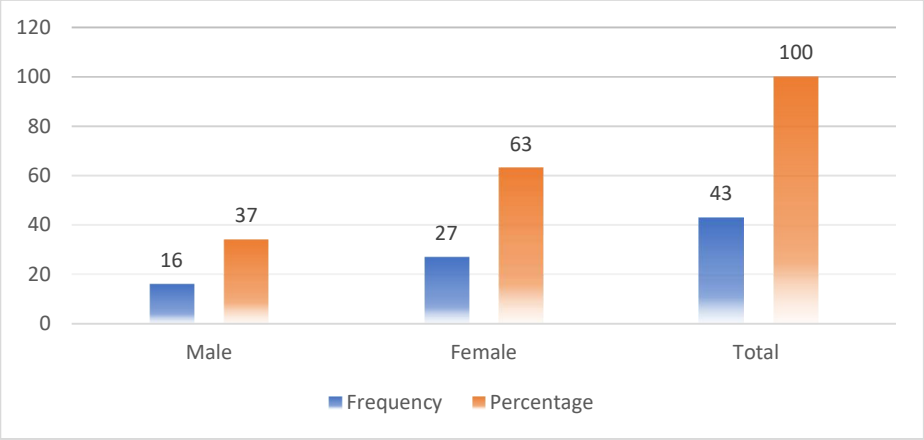

Figure 1: Gender-wise and distribution of *M. morganii*

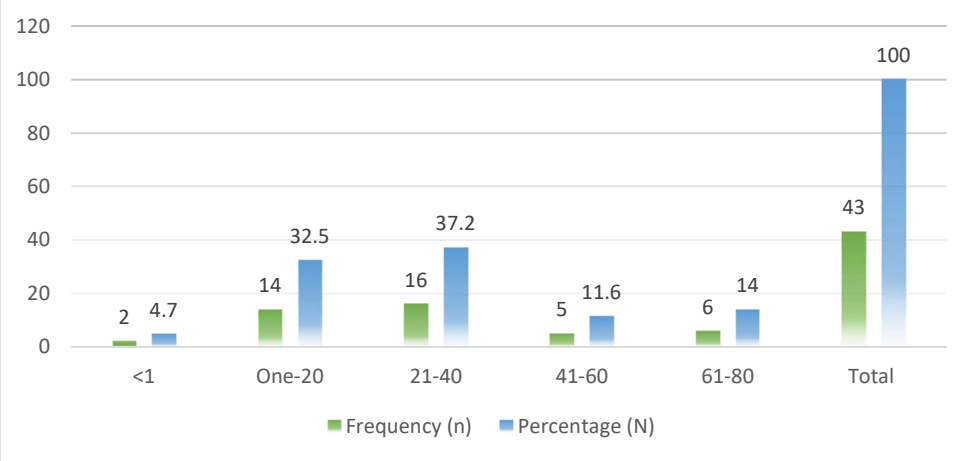

Figure 2: Age-wise distribution of *M. morganii*

The antibiotics susceptibility testing of isolates against 11 antibiotics was done via the disc diffusion method (**Table 3)**. The result revealed that high level of resistance against SXT, CIP, CEP, and CTX. While isolates of M. morganii are sensitive to FOS, AMC, TZP, MEM, IPM, AK, and CN.

Table 3: Antibiotic susceptibility pattern of *M. morganii* isolates (n=43)

	List of Antibiotics used	Resistant (%)	Sensitive (%)
1	CIP	29 (67%)	14 (33%)
2	SXT	33 (77%)	10 (23%)
3	FOS	4 (9%)	39 (91%)
4	AMC	11 (26%)	32 (74%)
5	TZP	3 (7%)	40 (93%)
6	CEP	28 (65%)	15 (35%)
7	CTX	25 (58%)	18 (42%)
8	MEM	3 (7%)	40 (93%)
9	IPM	7 (16%)	36 (84%)
10	AK	2 (5%)	41 (95%)
11	CN	12 (28%)	31 (72%)

The molecular detection of antibiotic resistance genes revealed that the *sul1* and *sul2* genes were present in sulfonamide (cotrimoxazole)-resistant isolates, while the *gyrA* and *gyrB* genes were identified in fluoroquinolone (ciprofloxacin)-resistant isolates (**Figures 3**). Among the cotrimoxazole-resistant isolates (n = 33) of *M. morganii*, the *sul1* and *sul2* genes were detected in 23 isolates. Similarly, among the ciprofloxacin-resistant isolates (n = 29),

the *gyrA* gene was detected in 11 isolates, and the *gyrB* gene was identified in 10 M. *morganii* (**Table 3**).

Table 1 Molecular detection of sulfonamide and fluoroquinolones resistant genes of *M. morganii*

	Antibiotic-resistant gene	Positive isolates	Percentage (%)
	Sulfonamide (cotrimoxazole) resistant isolates of *M. morganii* (n=33)		
1	*sul1*	23	69.6
2	*sul2*	23	69.6
	Fluoroquinolones (ciprofloxacin) resistant isolates of *M. morganii* (n= 29)		
3	*gyrA*	11	37.9
4	*gyrB*	10	34.4

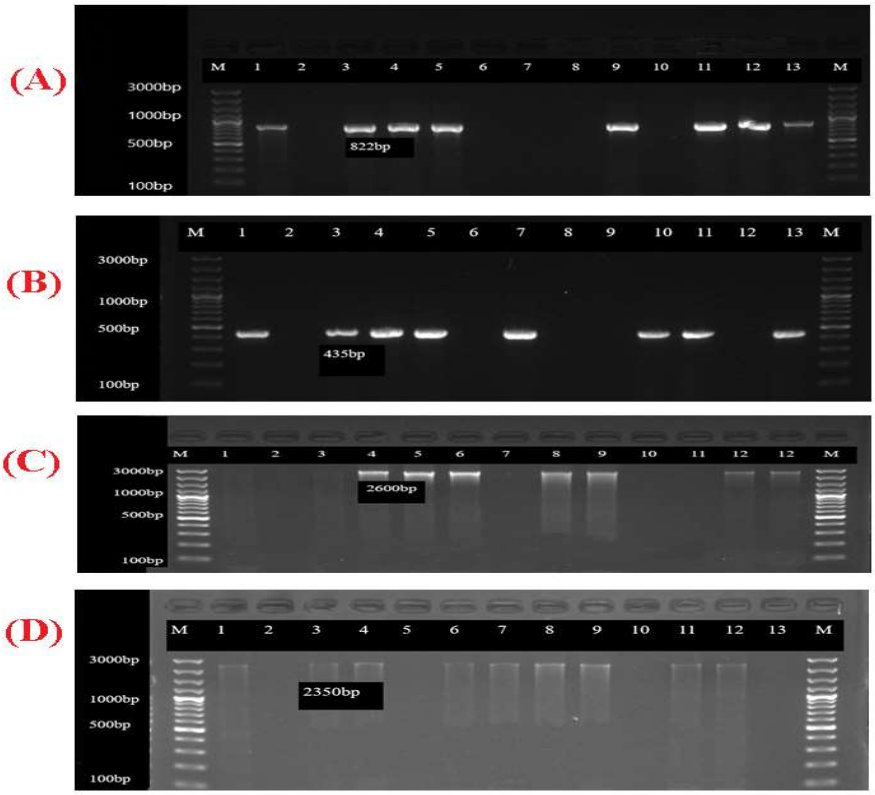

Figure 3: Gel electrophoresis of amplified PCR products, Lane M: DNA Ladder, Lane 1-3: Negative isolates, Lane 4-6: Positive isolates, Lane 7: Negative isolate, Lane 8-9: Positive isolate, Lane 10-11: Negative isolates, Lane 12-13: Positive isolate, Lane M: DNA Ladder **(A)** *sul*1 gene **(B)** *sul*2 gene **(C)** *gyr*A gene **(D)** *gyr*B gene.

The results of PCR showed that some of the isolates harbor different combinations of antibiotic-resistant genes. Seven isolates harbor all four resistant genes (*sul1, sul2, gyrA,* and *gyrB*), seven isolates had the combination of three genes (*sul1, sul2, gyrA* and *sul1, sul2, gyrB*) and 9 isolates had the combination of two genes (*sul1* and *sul2*) (**Figure 4).**

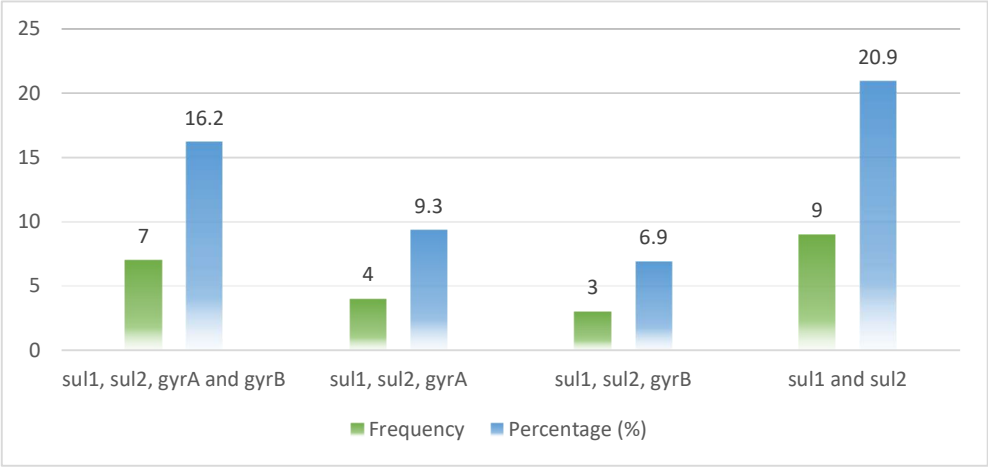

Figure 4: Combination of sulfonamide and fluoroquinolone resistance genes

After the detection of resistance genes in the isolates, PCR products were sent for sequencing. The aligned sequences of the genes were uploaded to the NCBI BLAST tool to check their similarity with sequences present in the database. After analysis, the results revealed that no mutations were detected in the genes, and they were 100% aligned with the sequences available in the database. The analysis and graphical representation of the data were performed using MS Excel software. The BioEdit software was used for the alignment of the oligonucleotide sequences. In the present research, no mutations were detected, and therefore, I-mutant software was not used.

4. Discussion

The study presented here investigates the genomic analysis of *M. morganii* strains from the South African region. The results indicated the presence of two highly multi-drug-resistant (MDR) strains isolated from the urine of infected patients. These isolates harbored resistance genes, including sul1, AmpC, and blaDHA-4, located on plasmids [27]. In another study, the antimicrobial resistance genes oqxB, fosA, floR, ble, mcr-9.1, fosA7, aadA2, aadA1, aph(6)-Id, aph(3'')-Ib, ant(2'')-Ia, blaTEM-1, qacEdelta1, tet(A), tet(B), sul1, and sul2 were identified in both environmental and clinical isolates, highlighting their significant role in antimicrobial resistance [28]. A separate study reported the prevalence of *M. morganii* in clinical urine samples from UTI patients, revealing that the predominant pathogens included *E. coli* (52.6%), *Proteus* (10.9%), *Klebsiella* (9.0%), *P. aeruginosa* (4.5%), *Citrobacter*, and *M. morganii* (13.5%) [29]. The same study indicated that 8.3% of urine samples were positive for *M. morganii* [30], while another investigation found the prevalence to be 1.87% [31]. The findings of the current study, in which 27.30% of clinical urine samples were positive for *M. morganii*, align with the trend of increasing recognition of *M. morganii* as an emerging uropathogen. A study reported the emergence of fluoroquinolone resistance genes, particularly gyrA and gyrB, in clinical isolates of *M. morganii* resistant to fluoroquinolones. The study revealed that these isolates exhibited high resistance to norfloxacin (512 µg/ml), ofloxacin and ciprofloxacin (256 µg/ml), and levofloxacin (64 µg/ml) [32]. In contrast, the present study detected the gyrA and gyrB genes in *M. morganii* isolates that were highly resistant to ciprofloxacin (67%). Another research investigation focused on the role of sulfonamide resistance genes (sul1 and sul2) in the outbreak of UTIs. In one such study, the PCR results revealed that a single *M. morganii* isolate harbored the sul1 gene [33], while another study detected sulfonamide resistance genes in one *M. morganii* isolate from UTI patients [34]. In the current study, PCR results

indicated that both the sul1 and sul2 genes were present in 23 isolates of *M. morganii*, with a combination of both genes found in 9 isolates. The findings from previous studies and the present research consistently emphasize the significant role of antimicrobial-resistant genes in resistance mechanisms against antibiotics. As observed, these antimicrobial-resistant genes are becoming increasingly prevalent within the *Enterobacteriaceae* family, further contributing to the global health burden.

5. Conclusions

Based on the findings of the present research, it is concluded that *M. morganii* is emerging as a multidrug-resistant uropathogen responsible for severe UTIs and exhibiting highly developed resistance mechanisms against major antibiotic families. The primary focus of this study was to investigate the antibiogram of *M. morganii* isolates collected from urine samples and to detect fluoroquinolone and sulfonamide antibiotic-resistance genes. The results indicate that the presence of *sul*1, *sul*2, *gyr*A, and *gyr*B genes plays a significant role in the development of resistance to fluoroquinolones and sulfonamide antibiotics.

Conflicts of Interest

The authors declare no conflicts of interest.

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