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Identification and antibiotics sensitivity of *Morganella morganii* isolated from urinary tract. infection. patients in Mosul, Iraq

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Abstract
The study was carried out on 70 urine samples were collected from 70 diagnosed urinary tract infection patients attended Ibn-Sena Educational Hospital, in Mosul City, during 5 months (November 2022 - March 2023). Samples were cultured on both MacConkey and Blood Agar. 48 (68.6%) of urine samples cultures were positive. for Gram negative. bacteria, whereas 22 (31.4%) for Gram positive bacteria. 4 (5.7%) isolates of *Morganella morganii* were isolated. Which identified by biochemical tests, VITEK -2 SYSTEM and confirm the identification with molecular techniques based on 16S rRNA. All isolates appeared resistance (100%) to Ampicillin, Ceftriaxone, Cefotaxime and Cefixime, whereas the resistance to Gentamycin and Doxycycline was (75%). Resistance to Meropenem, Imipenem and Levofloxacin was (50%). While Resistance to Ciprofloxacin was (25%).

Keywords: *Morganella morganii*, urinary tract infections

Introduction

Urinary tract infections (UTIs), the second-ranking infectious disease, are considered to be a serious global health issue. A urinary tract infection is when uropathogenic bacteria colonize the urinary system and cause different intensities of an inflammatory response. The clinical range is extremely diverse, varying from uncomplicated (pyelonephritis /. cystitis in young women.) to complicated UTIs affecting frail individuals (such as catheter-related UTIs, urinary diversion, transplant patients, the elderly population and patients with neurogenic bladders), which are frequently a cause of morbidity in these groups of people and carry a high chance of developing urosepsis. Numerous gram-negative and gram-positive bacteria can result in UTIs in both sexes, children, and adults (Foxman, 2014; Hussein *et al.*, 2018; Thattil and Santhosh, 2018) [9, 10, 26]

Although *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Staphylococcus spp. and Enterococcus spp. are the primary UTI-causing bacteria, *Morganella morganii* is frequently isolated from individuals who had UTI (Flores-Mireles *et.al*., 2015; Waller *et.al*., 2018) [8, 27].

M. morganii is a gram-negative rod. It is frequently found in soil, water and the gastrointestinal tracts of people and animals (Lin et al., 2015) [14]. According to Liu and his colleagues (2016), it is a significant opportunistic pathogen that is responsible for a variety of medical and community acquired illnesses.

M. morganii is a prevalent cause of UTIs, wound infections and septicemia. While it has sporadically been linked to pathologies in a variety of localizations, including brain abscesses, a liver infection, septic arthritis, pericarditis, peritonitis chorioamnionitis, rhabdomyolysis., after snakebites, fasciitis with necrosis develops, neonatal sulfhemoglobinemia, bilateral keratitis., in addition to nonclostridial gas gangrene, furthermore it isolated from infantile diarrheal cases (Al-Nakkar, 2000; Ponte and Costa, 2015; Liu *et al.*, 2016; Erlanger *et al.*, 2017; Imataki and Uemura, 2017; Minnullina *et al.*,2019; Shi *et al.*,2022) [2, 19, 15, 7, 11, 16, 25].

It is understood that uropathogenic *M. morganii* has many virulence factors: proteases, toxins and endotoxin, as well as fimbriae, flagella, ureases, hemolysin. Also, it has efflux pump, that is related to resistance to the main kinds of antibiotics (Liu *et al.*,2016; Minnullina *et al.*,2019; Behera *et al.*,2023) [15, 16, 4].

Due to the presence of the *amp*C resistance gene, *M. morganii* is natural resistance to the ampicillin, amoxicillin. and the majority of cephalosporins (first and second generation). The development of extensively drug-resistant (XDR) or multidrug-resistant (MDR) *M. morganii* as a result of the usage of broad-spectrum antibiotics caused therapy's failure in clinical situations. As a result, aminoglycosides, quinolones, co-trimoxazole or carbapenems are frequently suggested as treatments. However, it has become more and more clear that *M. morganii* has the ability to acquire additional resistance genes. Numerous clinical and animal sources have been identified to include highly resistant strains, according to recent investigations (Kohlmann *et al.*, 2018; Ryser *et al.*, 2021; Sharma *et al.*, 2021; Behera *et al.*, 2023) [12, 21, 24, 4].

M. morganii has been classified as a developing "superbug" due to its capacity to produce invasive disease, the existence of virulence factors and its potential to acquire resistance determining components (Bandy, 2020; Laupland *et al.*, 2022) [3, 13]

The aim of this study

The overall purpose of the study: is to highlight the relevance of *Morganella morganii* as an uropathogen and confirm its identification by molecular method based on 16S rRNA. Furthermore determination its resistant to the most common antibiotics.

Materials and methods

70 urine samples from UTI patients were collected from Ibn-Sena Educational Hospital, in Mosul City among

November 2022 and March 2023. For the isolation of UTI causing bacteria, loop full of urine sample was streaked on to MacConkey. Agar plate and Blood Agar plate, then incubated for 24hrs at 37° C. The following day, certain colonies were chosen and identified based on their morphological, cultural, and biochemical traits.

Identification

Bacterial isolates were identified based on bacteriological methods, such as characteristics and appearance of colonies on MacConkey agar and haemolysis on blood agar. Further identification was done by their gram stain reaction. Also, biochemical tests such as oxidase, catalase, Indole, Methyl red, Vogus Proskuar and Citrate utilization (IMViC), urease, phenylalanine deaminase, gelatinase, motility and H_2S generation tests were done. Then, bacterial isolates were identified by VITEK -2 SYSTEM (Bio-Merieux, France). Molecular methods based on 16S rRNA were used to confirm the identification of bacterial isolates (Rodriguez, 2021) [20].

DNA extraction

The Genomic DNA Extraction Kit (Geneaid Biotech, Taiwan) was used to extract the whole genomic DNA of bacterial isolates, according to the manufacturer's instructions. The extraction was done in Dept. of Biology-College of Science-University of Mosul.

DNA purification and quantification

Using a nano-drop spectrophotometer, the concentration and purity of the extracted DNA were determined (Cambridge CB4, England), at wave length 260-280 nm (Nishiguchi *et al*., 2002) [18].

16S rRNA gene amplification - polymerase chain reaction (PCR)

This reaction done according to (Abdulrazzaq & Faisal, 2022) [1] by using universal forward primer 27- F (5'-AGAGTTTGATCMTGGCTCAG-3') / and reverse primer 1552- R (5'- AAGGAGGTGATCCARCCGCA -3'), supplied by (Geneaid Biotech, Taiwan).

The produced mixture's final volume was 20 µl and it contained 10 µl of 1X *Go Taq* Green Master Mix, 4 µl of 1 ng/ l µl bacterial DNA, 2 µl of nuclease-free water, and 2 µl of 1 mM final concentration for F and R-Primer. The PCR amplification cycle's program and conditions are described in Table 1.

Table 1: Conditions of the PCR amplification cycle (Abdulrazzaq & Faisal, 2022) [1]

No	Steps	Temperature °C	Time	Cycle No.
1	First denaturation	95	3 min.	1
2	Denaturation	95	30 sec.	30
3	Annealing	54	30 sec.	
4	Elongation	72	90 sec.	
5	Last elongation	72	3 min.	1

Amplification products were electrophoresed for 45 minutes at 100 volts alongside normal DNA on a 1% (w/v) agarose gel. Gel was stained for one hour with ethidium bromide solution, followed by UV-transilluminator photography at 320 nm (provided by Royal Biotech GmbH-Germany) (Sambrook & Russell, 2001) [22].

16S rRNA gene sequence analysis

The 16S rRNA PCR results were transmitted to the Samogen Company in the USA for sequencing. In order to identify the tested isolates, the sequences of the samples were compared with the genes of the sequences at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), by identifying the tested isolates with the use of the Basic Local Alignment Search Tool (BLAST).

Antibiotic susceptibility testing

The test was carried out on Muller-Hinton Agar, following the recommendation given by CLSI depending on the Kirby-Bauer disc-diffusion method. The antibiotic agents used are described in table 2. the diameters of zones were measured by a ruler after 24hrs of incubation at 37˚C and were interpreted according to the CLSI recommendations (CLSI, 2021) [5].

Table 2: The antibiotics used for sensitivity test

No.	Antibiotics	Abbreviation	Concentration (µg)
1.	Ampicillin	AMP	30
2.	Meropenem	MEM	10
3.	Imipenem	IPM	10
4.	Ceftriaxone	CRO	10
5.	Cefotaxime	CTX	30
6.	*Cefixime*	CFM	5
7.	Gentamycin	CN	10
8.	Doxycycline	DO	10
9.	Ciprofloxacin	CIP	5
10.	Levofloxacin	LEV	*5*

Results and discussion

UTI results

From a total of 70 urine samples of UTI patients, 48 (68.6%) were positive for Gram negative bacteria, whereas 22 (31.4%) for Gram positive bacteria.

In our study, 4 (5.7%) isolates of *M. morganii* were recorded, according to the results of biochemical tests which revealed in (Table 3), and VITEK -2 SYSTEM.

All isolates produced β- hemolysis on blood agar, which appeared as a clear zone around colonies.

Table 3: Results of biochemical tests of *M. morganii*

Tests	Results
Oxidase	-
Catalase	+
Indole	+
Methyl red	+
Vogus Proskuar	-
Citrate utilization	-
Urease	+
Phenylalanine deaminase	+
Gelatinase	-
Motility	+
H_2S in TSI	-

Results of the 16S rRNA for 4 isolates were sent to Samogen Company in USA for sequencing. The sequences was analyzed at NCBI with using BLAST. The results of 4 isolates showed (98.29 %), (93.15%), (91.13%) and (90.15%), similarity ratio with references strains *M. morganii* A 18, NRLFFD293, NRLFFD293 and 27,

accession numbers MK027241.1, MN515398.1, MN515398.1and JX480503.1 respectively.

Table 4 displays the *M. morganii* resistance profile to the antibiotics being investigated. All isolates appeared resistance (100%) to Ampicillin, Ceftriaxone, Cefotaxime and Cefixime, whereas the resistance to Gentamycin and Doxycycline was (75%). Resistance to Meropenem, Imipenem and Levofloxacin was (50%). While Resistance to Ciprofloxacin was (25%). So these isolates were MDR.

Table 4: Resistance profile of *M. morganii* to the antibiotics

No	Antibiotics	Resistant (%)
1.	Ampicillin	100
2.	Meropenem	50
3.	Imipenem	50
4.	Ceftriaxone	100
5.	Cefotaxime	100
6.	Cefixime	100
7.	Gentamycin	75
8.	Doxycycline	75
9.	Ciprofloxacin	25
10.	Levofloxacin	50

Discussion

One of the most prevalent acquired bacterial infections is urinary tract infection (UTI), which affects people in the hospitals as well as community. The most common causative agent for both uncomplicated and complicated are Gram-negative (Flores-Mireles *et al.*, 2015) [8].

M. morganii has been discovered as a substantial cause of nosocomial infections., but in recent years, it has come to be seen as an increasingly serious pathogen. It was isolated from UTI patients in previous studies at (8.1%), (1.3%), (1.9%) and (1.8%) (Liu *et al.*, 2016) [15], (Naqid *et al.*, 2020) [17], (Dharati *et al.*, 2021) [6] and (Shi *et al.*, 2022) [25] respectively.

In our study, *M. morganii* showed highly resistant to many antimicrobial agents. Previous studies appeared that *M. morganii* resistant to Ampicillin (100%), Ceftriaxone and Gentamycin (14.3%), whereas it was sensitive to Imipenem, Ciprofloxacin and Levofloxacin (Naqid *et al.*, 2020) [17]. A study of Dharati (2021) and his colleagues detected that *M. morganii* resistant to Ampicillin and Cefixime (100%), Imipenem (88%), Ceftriaxone (86%), Gentamycin and Ciprofloxacin (68%), Levofloxacin (65%) and Meropenem (44%). Other study made by Rodriguez (2021) showed that this bacteria was resistant to Ampicillin, Imipenem, Ceftriaxone, Gentamycin, Doxycycline, Ciprofloxacin and Levofloxacin.

One of the most important virulence factors is antibiotic resistance. Drug resistance has also provided a significant issue for the prevention of bacterial infections since it has significantly accelerated the development and transmission of resistant genes in bacteria due to the intense selection pressure of commonly used antimicrobial agents (Liu *et al.*, 2016) [15].

MDR, XDR, and pan-drug resistant phenotypes in bacterial isolates are becoming more common. It is evident from this study that uropathogens are evolving resistance to the most popular antibiotics used to treat simple UTIs. Major factors known to affect the evolution and transmission of multidrug resistance among microorganisms include incomplete doses., ease of access., overprescribing. prescribing higher generation antibiotics., prescribing antibiotics without laboratory results, and indiscriminate use of antibiotics in the agriculture and livestock sectors. Considering that drug resistance is primarily an acquired trait that can be lost at any time. Because of this, the resistance profile of various drugs frequently exhibits increases and decreases over time toward a particular pathogen (Sanjee *et al.*, 2017; Laupland *et al.*, 2022) [23, 13].

Conclusion

Due to its scarcity and poor possibility for nosocomial outbreaks, *M. morganii* previously received little attention. owing to the bacteria's induced resistance to numerous antibiotics especially β-lactam antibiotics, *M. morganii* infections are particularly concerning epidemiologically. *M. morganii* is a rare medical opportunistic infection, however, this significant pathogen can't be ignored. To identify M. morganii's pathogenic mechanism and stop the spread of Morganella infections, more research is required.

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