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Microbial Diversity of Urinary Tract Infection of Some Egyptian Patients In Suez Governorate Area

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ABSTRACT

Urinary tract infections (UTIs) are one of the most common infectious illnesses that afflict people, they pose a serious threat to public health and place a heavy financial burden on society. Antibacterial resistance of Enterobacteriaceae, especially the principal uropathogens *Escherichia col*i and *Klebsiella pneumoniae*, has dramatically developed globally as a result of the high empiric use of antibiotics for the treatment of UTI, the recovered microbial isolates were divided into 88-gram negative bacteria (88 isolates), gram positive ones (only 8 isolates) and4 yeast isolates. Our results include fungal isolates three as *Candida tropicalis* and only one was *Candida dubliniensis*; the rest of one hundred isolates which represent eighteen bacterial isolates clarified as Gram-negative Bacteria which contain *E. coli* with ratio 46(44%), *Klebsiella pneumoniae* 24 (25%), *Pseudomonas aeruginosa* 10(10%), *Enterobacter cloacae* 2(2%) and *Acinetobacter baumannii, Citrobacter koseri, Serratia liquefaciens, Serratia Plymuthica, Sphingomonas paucimobilis, Morganella morganii* with 1(1%) for each of them ,and Gram-positive bacteria watch clarified as follows, *Staphylococcus lentus* and, *Staphylococcus haemolyticus* with ratio 2(2%) for each them and *Staphylococcus xylosus, Enterococcus faecalis, Staphylococcus saprophyticus* and *Enterococcus sp* with 1 % for each of them. All isolates were tested against different antibiotics in order to determine their antibiotic resistance pattern. Among one hundred isolates many isolates were MDR (72%), After conducting statistical operations, the samples were selected are (2, 21, 38, 45 and 87) as the most resistant isolates to antibiotics

Keywords: Urinary tract infections, illnesses, Antibacterial, Egyptian patients,

1. Introduction

Bacterial infection remains one of the most critical threats to human health, especially in third world countries (Yestrepsky *et al.*, 2013).

UTI is a common health problem in both community and nosocomial settings. UTI is among one of the most common infections occurring particularly in women. As reported by the National Ambulatory Medical Care Survey, UTI alone is responsible for nearly seven million patient visits in outpatient department (OPD) as well as up to one million visits in hospital emergency department, resulting in about 100,000 hospitalizations (Foxman, 2010). Nearly 50–60% of all women suffer from an episode of UTI at least once in their lifetime (Obstetricians and Gynecologists, 2008). If the predisposing factors which are responsible for the occurrence of UTI are not timely diagnosed and treated, then it is also common for UTI episodes to reoccur(Foxman *et al.*, 2000).

Untreated UTI can result in serious complications such as kidney damage, renal scarring, and renal failure(Stoller *et al.*, 2004; Momoh *et al.*, 2011).

UTI causative bacteria in humans, *E. coli* is the main cause of UTI, enteric, extra-intestinal and systemic infections worldwide. The *Uropathogenic E. coli* (UPEC) predominantly causes nosocomial (30-50%) and community acquired (80-90%) UTIs. The drug resistance in *Uropathogenic E. coli* is

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spreading globally which is an alarming situation. The transmission of antibiotic resistance among uropathogens causing UTIs is life threatening worldwide(Azam *et al.*, 2022).

UTI usually starts as a bladder infection (cystitis), but can develop to acute kidney infection (pyelonephritis), ultimately resulting in scaring and renal failure. UTI is also a major cause of sepsis, which has a mortality rate of 25% and results in more than 36,000 deaths per year in the USA(Stamm and Norrby, 2001).

In the world, the unusual development and increasing of multidrug-resistant in bacterial pathogens is a great health problem which is rising day by day in a growing community(Luzzaro *et al.*, 2009; D'Andrea *et al.*, 2011). In worldwide, for the last two or three decades, hospital acquired UTIs are increased due to the emergence of multidrug resistant uropathogens (Moges *et al.*, 2002; Barlow and Nathwani, 2005). Bacterial resistance, one of the biggest threats to human health in the 21st century, is the ability of bacterial cells to resist one or more types of antibiotics (Khandelwal *et al.*, 2019).

Over the last decade, whole-genome sequencing (WGS) has been identified as one of the most promising techniques in clinical microbiology (Didelot *et al.*, 2012; Inouye *et al.*, 2014). Although its use in clinical microbiology increases, WGS is differentially implemented depending on the pathogen or the intended uses. (Loman *et al.*, 2012; Tang *et al.*, 2017).

Advanced genomics and proteomics technologies will continue to play a critical role in bacterial identification and characterization in the 21st century (Emerson *et al.*, 2008).

2. Materials and Methods

2.1. Sample Collection

Clean catch midstream urine was collected from each patient into a 20mL calibrated sterile screw-capped universal container which was distributed to the patients. The specimens were labeled, transported to the laboratory, and analyzed within 6 hours. In each container boric acid (0.2mg) was added to prevent the growth of bacteria in urine samples. All patients were well instructed on how to collect sample aseptically prior to sample collection to avoid contaminations from urethra. The study was conducted after due ethical approval which was subjected to the hospital administrations.

2.2. Isolation of clinical samples:

The urine samples of 100 patients, comprised of 29 males and 71 females, who attended to the outpatient departments (OPDs) and in-patient departments of Suez Canal Authority hospital during over ten months' period (May 2021 – March 2022) and had clinical evidence of urinary tract infection, determined by urologists, were included in this study. The age of patients included in the study ranged from 15 to ≥60 years. patients who on antibiotic therapy were excluded from the study.

2.3. Sample processing

A calibrated loop method was used for the isolation of bacterial pathogens from urinary samples. A sterile 4.0mm platinum wired calibrated loop was used which delivered 0.001mL of urine. A loopful urine sample was plated on nutrient agar as standard for isolation and purification one inoculation on Cystine-Lactose-Electrolyte Deficient (CLED) agar, MacConkey agar and blood agar medium (Mahon *et al.*, 2018).The inoculated plates were incubated at 37∘C for 24 h and for 48 h in negative cases. The number of isolated bacterial colonies was multiplied by 1000 for the estimation of bacterial load/mL of the urine sample. A specimen was considered positive for UTI if an organism was cultured at a concentration of ≥105 cfu/mL or when an organism was cultured at a concentration of 104 cfu/mL and >5 pus cells per high-power field were observed on microscopic examination of the urine (Mahon *et al.*, 2018). Each urine sample with significant bacteria growth was sub-cultured to obtain pure isolates and the isolates were subjected to biochemical tests for identification.

2.4. Culture media

Different types of enrichment media and selective media were used for isolation and identification of bacteria. The plates were incubated aerobically at 37OC for optimal bacterial growth. Pure colonies were selected, coded and tested against antibiotics through agar disc diffusion method according to Hombach-Klonisch *et al.* (2018). The chemical constituents of these media are as following.

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2.4.1. Blood Agar media

According to Farrell and Robinson, (1972), blood agar tested in animals with improved nutritional properties, suitable for the cultivation of demanding pathogens and other microorganisms, with the following composition (g / l): 15 protease peptone, 2.5 hepatic digestion, 5 yeast extract, 5 sodium chloride (NaCl) and 15 agars per 1 liter of D. H2O. The final pH was 7 (at 25 ° C). Dissolve by boiling and sterilize in an autoclave at 121 ° C for 15 minutes. After cooling to 4550 ° C (7%) sterile blood was added.

2.4.2. MacConkey Agar

MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. This medium consists of the following components (g / l): 17 peptones, 3 peptone protease, 10 lactose, 1.5 bile salts, 5 sodium chloride (NaCl), 0.03 neutral red, 0.001 crystal violet and 13.5 agar. The final pH was 7.1. Dissolve by boiling and sterilize in an autoclave at 121 ° C for 15 minutes (Downes and Ito, 2001).

2.4.3. CLED agar

CLED agar is a differential, nonselective culture medium, which, due to the inclusion of cystine, supports the growth of the vast majority of potential urinary pathogens, facilitates the recognition and easy differentiation of colony morphology as well as of the ability to ferment lactose, and has the additional advantage, due to its electrolyte deficiency, of preventing the swarming of Proteus spp (Arneil *et al.*, 1970).This medium consists of the following components Formula* Per Liter Purified Water Pancreatic Digest of Gelatin 4.0 g Pancreatic Digest of Casein 4.0 Beef Extract 3.0 Lactose 10.0 L-Cystine 0.128 Bromthymol Blue 0.02 Agar 15.0 pH 7.3 +/- 0.2(LISTO).

2.4.4. Mueller-Hinton Agar

This medium is used when testing for antimicrobial susceptibility by the disk diffusion method. This formula corresponds to the Clinical and Laboratory Standard Institute (2020), this medium contains few sulfonamide, trimethoprim and tetracycline inhibitors and offers satisfactory growth of most non-fastidious pathogens (CLSI 2020). This medium consists of the following ingredients (g / l): beef extract 300 (infusion form), hydrolyzed casein 17.5, starch 1.5 and agar 17. Final pH was 7.3 Preparation: The components were added to 1.0 liter of distilled water and mixed thoroughly. The medium was carefully heated to boiling. It was autoclaved for 15 min at a pressure of 15 psi at 121 ° C and poured into sterile Petri dishes (Mueller *et al.*, 1941).

2.5. Purification of bacterial isolates:

The purification process of the examined bacterial isolates was carried out using the agar plate method. All colonies of different shapes and colors that showed separate growth on nutrient agar medium were collected and spread again on the agar surface of plates containing the same isolation medium. At the end of the incubation period, only growth that appeared as a single separate colony of different shape and color was harvested and re-seeded onto the surface of the agar plate in the isolation medium for several consecutive times to ensure its purity. Purity was checked microscopically and morphologically using individual and Gram stains. Only pure isolates were sub-cultured on slant nutrient agar medium and stored at 45 ° C for future research. The purified colonies were prepared for use in identification and other studies.

2.6. Morphological, Physiological and Biochemical tests for Identification of bacterial isolates.

2.6.1. Stains

2.6.1.1. Simple stain was prepared and stained according to Barrow *et al.*, (1993) which include Methylene blue, crystal violet, and carbolfuchsin.

2.6.1.2. Gram's reaction

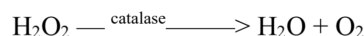
Gram's stain method was given by (Gram, 1884). Total bacterial isolates were classified on the basis of Gram reaction to Gram positive and Gram negative, depending on the ability of bacterial cells staining.

Solutions:

a) 2g Crystal violet dissolved in 20ml Ethanol.
b) 0.8g Ammonium oxalate dissolved in 80ml Distilled Water Mix solution (a) with (b).
c) 1g Iodine +2g KI dissolved in 300 ml Distilled water
d) 2.5% Safranin stain in 95% ethanol.
All solutions up to 100 ml through adding Distilled water (Aryal, 2018).

2.6.2 Catalase activity (Benson, 1967).

Most aerobes and facultative anaerobes that utilized oxygen produce hydrogen peroxide, which is toxic to their own enzyme systems. Their survival in the presence of this metabolite is possible because they produce an enzyme called catalase, which converts the hydrogen peroxide to water and oxygen as the following equation:



$$H_2O_2 \xrightarrow{catalase} H_2O + O_2$$

Presence of catalase enzyme is considered the main differentiator between Staphylococcus sp. positive catalase and Streptococcus sp. negative catalase. Isolated bacterial colony was taken by straight wire loop and mixed with 30 % H2O2 in a clean glass tube. Catalase positive showed clear bubbles that indicated the presence of catalase enzyme in bacteria.

2.6.3 Slide and tube coagulase test

The tested bacterial suspension was treated with a drop of citrated plasma and mixed well. Agglutination or clumping of cocci within 5- 10 seconds was taken as positive. Some strains of *S. aureus* may not produce bound coagulase, and such strains must be identified by tube coagulase test. Three test tubes were taken and labeled " test ", " negative control " and " positive control ". Each tube was filled with 0.5 ml of 1:10 diluted plasma. 0.1 ml of overnight broth culture of *S. aureus* was added to the tube labeled test. 0.1 ml of overnight broth culture of known *S. aureus* was added to the tube labeled positive control, and 0.1 ml of sterile broth was added to the tube labeled negative control. All the tubes were incubated at 37 ° C and observed up to four hours. Positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube. The tube is kept at room temperature for overnight incubation if the test remains negative until four hours at 37 °C.

2.6.4. Oxidase Test (Aryal, 2018b).

The oxidase test detects the presence of a cytochrome oxidase system that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye- tetramethyl-p-phenylene-diamine. The dye is reduced to deep purple color. This test is used to assist in the identification of Pseudomonas, Neisseria, Alcaligens, Aeromonas, Campylobacter, Vibrio, Brucella and Pasteurella, all of which produce the enzyme cytochrome oxidase (Aryal, 2018b).

2.6.5 Lactose fermentation test (Andrews *et al.*, 2018).

MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. Pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium. Neutral red is a pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. Agar is the solidifying agent

2.7. Automated Identification by using the Biomerieux VITEK2 system

The VITEK2 system is an automated microbial identification system that provides highly accurate and reproducible results as shown in multiple independent studies. In this thesis all biochemical analysis by this device were performed in Suez Canal Authority hospital laboratory. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK2 system offers a state-of-the-art technology platform for phenotypic identification methods. The reagent cards have 64 wells that can each Contain an individual test substrate. Substrates measure various metabolic activities

such as acidification, alkalization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. There are currently four reagent cards available for the identification of different organism classes as follows:

2.8. Antibiotics assay

The antimicrobial susceptibility of isolates was done to determine MDR ones between total isolates. Antibiotic sensitivity test was done through disc diffusion method on Muller Hinton agar, plates, the panels of different Twenty-Five (25) specificity for each genes of isolates, for example member's genes of Staphylococcus were tested against eleven antibiotics (CN: Gentamicin, E: Erythromicin, MXF: Moxifloxacin, RD: Rifampicin, CIP: Ciprofloxacicn, SXT: Trimethoprim/Sulfamethoxazol, LZD: Linezolid, DA: Clindamycin, TE: Tetracyclin, TEC: Teicoplanin).

Antibiotics included the following: AMP: Ampicillin, SAM: Ampicillin/ Sulbactam, TZP: Piperacillin/ Tazobactam, FOX: Cefoxitin, CAZ: Ceftazidime, CRO: Ceftriaxon, FEP: Cefepime, MEM: Meropenem, AK: Amikacin, CN: Gentamicin, TOB: Tobramycin, CIP: Ciprofloxacin, F: Nitrofurantoin, SXT: Trimethoprim/ Sulfamethoxazole, ATM: Aztreonam, ETP: Ertapenem, IPM: Imipenem, E: Erythromicin, MXF: Moxifloxacin, RD: Rifampicin, LZD: Linezolid, DA: Clindamycin, TE: Tetracyclin, TEC: Teicoplanin, LEV: Levofloxacin.

Bacterial cultures in nutrient solution were prepared overnight for each test and 15 ml Mueller Hinton agar plates were prepared and then surface dried for 30 minutes at 37 °C, 250 µl of bacterial culture was spread on the surface of the dried agar plates using a sterile glass spreader and allowed to absorb on the agar for 10 minutes. Antibiotic discs placed on the surface of the agar plate, kept at 20oC for 10 min for facilitating antibiotic diffusion through agar layer then incubated at 37 °C for 24 hours. Antimicrobial activity was determined by measuring the diameter of the inhibition zone in mm then take the mean measurement of three replicate plates for each isolate (Vaghasiya and Chanda, 2010)**.** Susceptibility and resistance diameters of tested isolates against different 18 antibiotics were compared with CLSI standard table 2020.

2.9. Genomic identification of the most extensive MDR isolates

2.9.1. PCR Amplification of DNA Sequencing 16S rDNA.

Two isolates' pure cultures were established in Luria Bertani (LB) broth, and the methodology by (24) for obtaining genomic DNA was followed (Sambrook *et al.*, 1989). The universal bacterial primers F 5'- AGA GTT TGA TCC TGG CTC AG-3' and R 5'- GGT TAC CTT GTT ACG ACT T-3 from Sigma Scientific Services Co. were used for the PCR amplification of the 16S rRNA gene. Maxima Hot Start PCR Master Mix (2X) 25 l, 20 mol of each primer, in a reaction volume of 50 l. The following conditions were used in the PCR reaction: first cycle denaturation at 95 °C for 10 min, followed by 35 cycles of 95 °C for 30 sec, 65 °C for 1 min, 72 °C for 1 min, and finally the last cycle with extension at 72 °C for 10 min.

2.9.2. Purification of PCR Product and 16S rRNA Gene Sequencing.

Amplified DNA products were purified using the GeneJET™ PCR Purification Kit (Thermo) as follows: 45 µl of Binding Buffer was thoroughly mixed with the PCR product mixture, the mixture was transferred to a GeneJET™ Purification Column, centrifuged at 14,000 xg for 1 minute, and the flow-through was discarded. Add 100 µl of Wash Buffer to the GeneJET TM Purification Column, centrifuge for 1 minute, and discard the flow-through. Finally, the purified PCR products were eluted using 25 µl of elution buffer, centrifuged at 14000 rpm for 1 min, and the eluted DNA was stored at -20°C until use. Purified DNA products were sequenced by capillary electrophoresis using an ABI 3730xl DNA sequencer (GATC Company). Perform sequence data analysis using Sequencing Analysis Software

3. Results

One hundred mid-stream urine samples were collected from 71 females and 29 males (Figure 1).

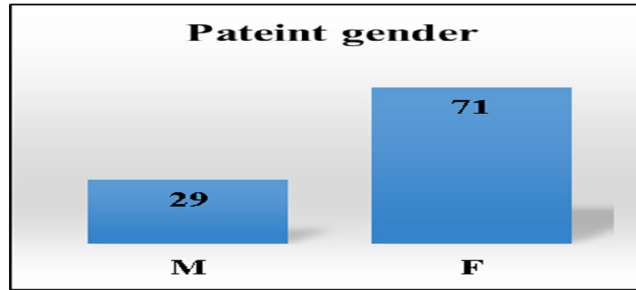


Fig. 1: Patient gender

Source of samples was divided into the outpatient departments (OPDs) (64%) and in-patient departments (36%) of Suez Canal Authority hospital during over 8 months' period (may 2021 – March 2022) and had clinical evidence of urinary tract infection (Figure 2).

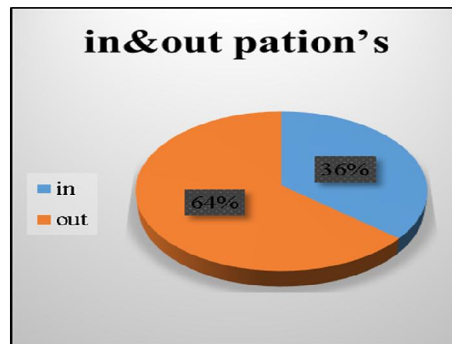


Fig. 2: Count of in& out patients.

The recovered microbial isolates were divided into 88-gram negative bacteria (88 isolates), -gram positive ones (only 8 isolates) and4 yeast isolates (Figure 3) (Table 1).

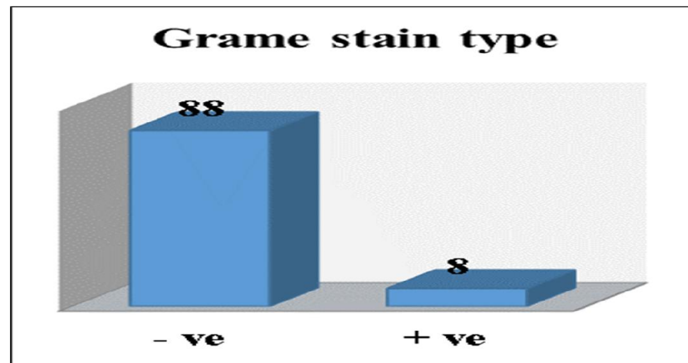


Fig. 3: Gram stain type

Table 1: Demographic characteristics of Patients

Variable	n (%)
Gender	
Male	29(29%)
Female	71(71%)
Age	
> 30 years	15(15%)
- 60 years	34(34%)
> 60 years	51(51%)

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In & Out patient's	
In	36(36%)
Out	64(64%)
Gram reaction type	
G +ve	8(8%)
G –ve	88(88%)
Yeast	4(4%)
Total	**100(100%)**

Isolates cultivated on blood agar and exhibit different type of hemolysis represented in Figure (4); 65 % isolates without blood hemolysis activity followed by 25 % isolates beta hemolysis and finally 9 (10%) where alpha hemolysis isolates, these results clarified in Figure (4).

Within gram negative isolates; 70 isolates (80%) were lactose fermenting isolates while 17 isolates (20%) where lactose non-fermenting isolates Figure (5). In the blood agar we obtained about 2(6%) isolates of gram-positive beta hemolysis, nothing (0%) of gram-positive alpha hemolysis, 22 (65%) isolates of gram-negative beta hemolysis and 10(29%) isolates of gram-negative alpha hemolysis.

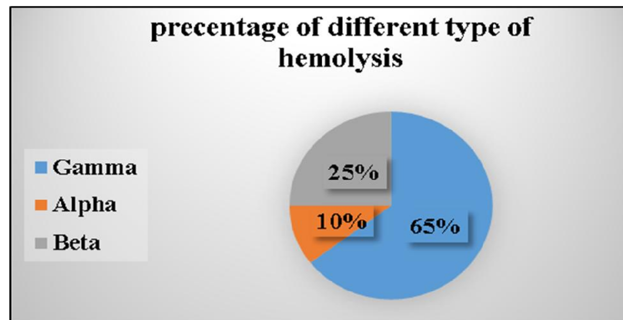

Fig. 4: Percentages of different types of hemolysis of one hundred isolates

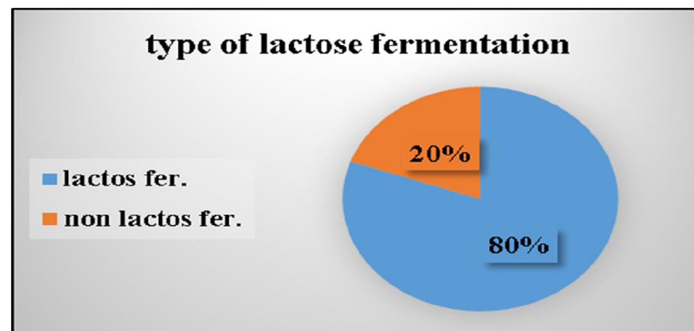

Fig. 5: Type of lactose fermenting.

Isolates cultivated on CLED agar and after doing biochemical analytics we obtained about 6 isolates positive to catalase test, 2 isolates positive to coagulase test and finally there are 4 isolates from candida species Figure (6).

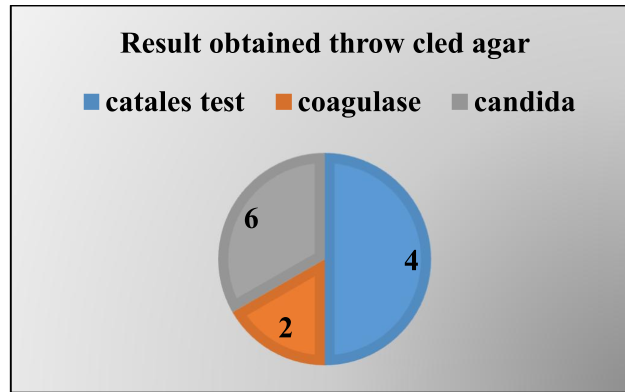

Fig. 6: Result obtained throw cled agar

Standing on Vitek report of biochemical analysis of one hundred isolates; isolates belonging to different 5 genera and species; Out of one hundred isolates tow isolates defined as fungal isolates belonging to candida species which represent 3 isolates *Candida tropicalis* and only one was *Candida dubliniensis;* the rest of one hundred isolates which represent 18 bacterial isolates their genera and species with ratios clarified in Table (2) and Figure (7).

Table 2: Frequency of bacterial isolates of UTI patients

Microorganism	n(%)
Gram-negative Bacteria	
E. coli	46(44%)
Klebsiella pneumoniae	24(25%)
Pseudomonas aeruginosa	10(10%)
Acinetobacter baumannii	1(1%)
Enterobacter cloacae	2(2%)
Citrobacter koseri	1(1%)
Serratia liquefaciens	1(1%)
Serratia Plymuthica	1(1%)
Sphingomonas paucimobilis	1(1%)
Morganella morganii	1(1%)
Gram-positive bacteria	
Staphylococcus lentus	2(2%)
Staphylococcus haemolyticus	2(2%)
Staphylococcus xylosus	1(1%)
Enterococcus faecalis	1(1%)
Staphylococcus saprophyticus	1(1%)
Enterococcus spp	1(1%)
Yeast	
Candida tropicalis	3(3%)
Candida dubliniensis	1(1%)

n =number

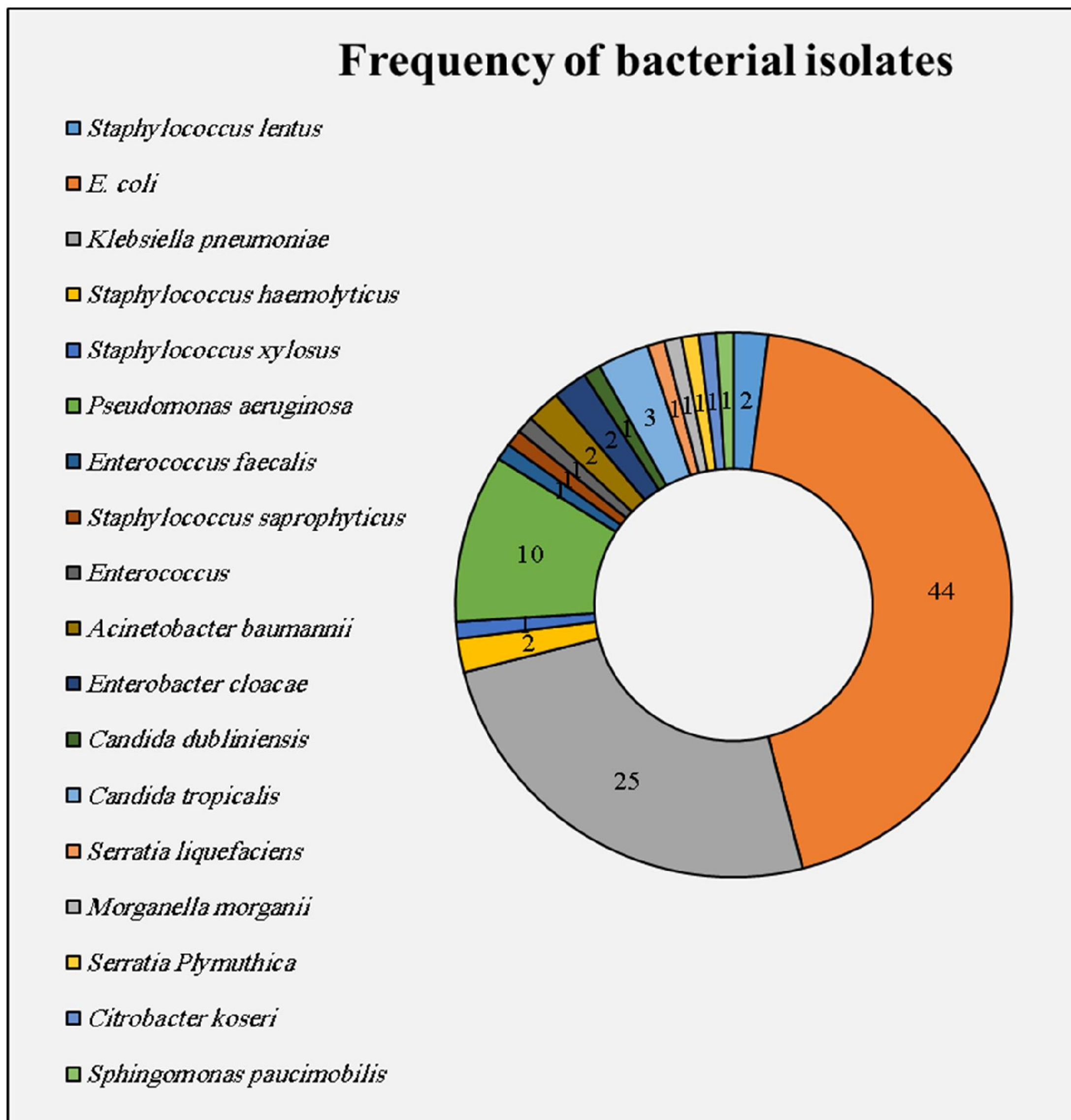

Fig. 7: Genera frequency of bacterial isolates

Antibiotic susceptibility test

All isolates were tested against different groups antibiotics depending on type of genus and species of the tested isolates that summarized in the following tables (3, 4, 5, 6 ,7, and 8)

In the genus *Staphylococcus*, 6 isolates were isolated from patients with urinary tract infections, and antibiotics were selected for them against 10 antibiotics according to the CLSI 2020, and the resistance ratios were as follows *S. lentus (70%), S. haemolyticus (30%), S. haemolyticus (30%), S. xylosus (90%), S. lentus (20%), S. saprophyticus (30%).*

In the genus *Enterococcus*, 2 isolates were isolated from patients with urinary tract infections, and antibiotics were selected for them against 8 antibiotics according to the CLSI 2020, and the resistance ratios were as follows, *E. faecalis (71.42%), Enterococcus spp (42.85%).*

In the genus *Acinetobacter*, one isolates were isolated from patients with urinary tract infections, and antibiotics were selected for them against 12 antibiotics according to the CLSI 2020, and the resistance ratios were as follows *A. baumannii (83.33%).*

Antibiotic tests were conducted on 76 isolates of *Enterobacteriaceae & Yersiniaceae* that were isolated from samples taken from patients with urinary tract infections, and the results were as shown in the following table (7)

Table 3: Antibiogram of *Genus: Staphylococcus isolated* from UTI infected patients (n=6) against 10 antibiotics.

Antibiotics names	Symbols	Sensitive (n)	Percentage (%)	Resistant (n)	Percentage (%)
Gentamicin	CN	5	83.33	1	16.66
Erythromicin	E	0	0	6	100
Moxifloxacin	MXF	4	66.66	2	33.33
Rifampicin	RD	4	66.66	2	33.33
Ciprofloxacicn	CIP	4	66.66	2	33.33
Trimethoprim/ Sulfamethoxazol	SXT	3	50	3	50
Linezolid	LZD	5	83.33	1	16.66
Clindamycin	DA	2	33.33	4	66.66
Tetracyclin	TE	1	16.66	5	83.33
Teicoplanin	TEC	4	66.66	2	33.33

Table 4: Antibiogram of *Genus: Enterococcus* isolated from UTI infected patients (n=2) against 8 antibiotics.

Antibiotics names	**Symbols**	**Sensitive (n)**	**Percentage (%)**	**Resistant (n)**	**Percentage (%)**
Gentamicin	CN	2	100	0	0
Erythromicin	E	0	0	2	100
Rifampicin	RD	1	50	1	50
Ciprofloxacicn	CIP	0	0	2	100
Linezolid	LZD	1	50	1	50
Tetracyclin	TE	0	0	2	100
Teicoplanin	TEC	2	100	0	0

Table 5: Antibiogram of *Genus: Acinetobacter* isolated from UTI infected patients (n=1) against 12 antibiotics.

Antibiotics names	**Symbols**	**Sensitive (n)**	**Percentage (%)**	**Resistant (n)**	**Percentage (%)**
Ampicillin/ Sulbactam	SAM	0	0	1	100
Piperacillin/ Tazobactam	TZP	0	0	1	100
Ceftazidime	CAZ	0	0	1	100
Ceftriaxon	CRO	0	0	1	100
Cefepime	FEP	0	0	1	100
Meropenem	MEM	0	0	1	100
Amikacin	AK	1	100	0	0
Gentamicin	CN	0	0	1	100
Tobramycin	TOB	0	0	1	100
Ciprofloxacin	CIP	0	0	1	100
Levofloxacin	LEV	0	0	1	100
Imipenem	IPM	1	100	0	100

Table 6: Antibiogram of Family*: Enterobacteriaceae & Yersiniaceae* isolated from UTI infected patients (n=76). against 18 antibiotics.

Antibiotics names	Symbols	Sensitive (n)	Percentage (%)	Resistant (n)	Percentage (%)
Ampicillin	AMP	9	11.84	67	88.15
Ampicillin/Sulbactam	SAM	18	23.68	58	76.31
Piperacillin/Tazobactam	TZP	50	65.78	26	34.21
Cefoxitin	FOX	47	61.84	29	38.1
Ceftazidime	CAZ	50	65.78	26	34.21
Ceftriaxon	CRO	30	39.47	46	60.52
Cefepime	FEP	28	36.84	48	63.15
Meropenem	MEM	48	63.15	28	36.84
Amikacin	AK	61	80.26	15	19.73
Gentamicin	CN	52	68.42	24	31.57
Tobramycin	TOB	45	59.21	31	40.78
Ciprofloxacin	CIP	34	44.73	42	55.26
Levofloxacin	LEV	54	71.05	22	28.94
Nitrofurantoin	F	49	64.47	27	35.52
Trimethoprim/Sulfamethoxazole	SXT	33	43.42	43	56.57
Aztreonam	ATM	35	46.05	41	53.94
Ertapenem	ETP	54	71.05	22	28.94
Imipenem	IPM	65	85.52	11	14.47

Table 7: Antibiogram of Family*: Enterobacteriaceae & Yersiniaceae*

Code	Species	Resistant to (n)(%)	Antibiotics Symbols
2	***E. coli***	15 (83.33%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, MEM ,AK, TOB ,CIP, LEV, SXT, ATM, ETP
3	***K. pneumoniae***	10 (55.55%)	AMP, SAM, CAZ, CRO, FEP, TOB, CIP, LEV, F, SXT,
5	***K. pneumoniae***	15 (83.33%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, MEM, AK, CN, TOB, CIP, LEV, F, SXT
6	***E. coli***	8 (44.44%)	AMP, SAM, TZP, FOX, CAZ, CRO, F, SXT
7	***E. coli***	12 (66.66%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, MEM, TOB, CIP, LEV, SXT
8	***E. coli***	7 (38.88%)	AMP, CAZ, CRO, FEP, CIP, LEV, SXT
12	***K. pneumoniae***	3 (16.66%)	AMP, TZP, F
13	***K. pneumoniae***	10 (55.55%)	AMP,SAM,FOX,CAZ,CRO,FEP,CN,TOB, CIP, LEV
14	***E. coli***	10 (55.55%)	AMP, SAM, FOX, CAZ, CRO, FEP, CN, TOB, CIP, ATM
15	***K. pneumonia***	13 (72.22%)	AMP, SAM, FOX, CRO, FEP, MEM, TOB, CIP, LEV, F, SXT, ATM, ETP
16	***E. coli***	10 (55.55%)	AMP, SAM, TZP, CAZ, CRO, FEP, LEV,F, ATM, ETP
17	***E. coli***	14 (77.77%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP ,AK, TOB, CIP, LEV, F, ATM, ETP
18	***E. coli***	4 (22.22%)	AMP, SAM, TOB, ETP
19	***E. coli***	5 (27.77%)	AMP, SAM, TOB, SXT, ETP
23	***E. coli***	9 (50%)	AMP, CAZ, CRO, FEP, CN, TOB, CIP, LEV, SXT
24	***E. coli***	5 (27.77%)	AMP, SAM, MEM, AK, TOB,
25	***K. pneumoniae***	2 (11.11%)	AMP, F
28	***K. pneumoniae***	2 (11.11%)	SXT ,MEM
29	***E. coli***	4 (22.22%)	AMP, SAM, MEM, AK,
30	***K. pneumoniae***	10 (55.55%)	AMP, SAM, CAZ, CRO, FEP, MEM, CIP, SXT, ATM, ETP
31	***K. pneumoniae***	15 (83.33%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, MEM, CIP, LEV, F, SXT, ATM, ETP, IPM
32	***K. pneumoniae***	11 (61.11%)	AMP, SAM, TZP, FOX, FEP, CN, TOB, CIP, LEV, SXT, ETP

Code	Species	Resistant to (n)(%)	Antibiotics Symbols
33	***E. coli***	11 (61.11%)	SAM, TZP, FOX, CAZ, CRO, FEP, CN, TOB, CIP, LEV, SXT
35	***E. coli***	2 (11.11%)	SAM, MEM
36	***E. coli***	6 (33.33%)	AMP, SAM, FOX, FEP, CN, ATM
37	***E. coli***	9 (50%)	AMP, SAM, FOX, CAZ, CRO, FEP, AK, SXT, ATM
40	***E. coli***	5 (27.77%)	AMP, SAM, TZP, MEM, ATM
41	***K. pneumoniae***	7 (38.88%)	AMP, SAM, TZP, FEP, MEM, F, ATM
42	***E. coli***	12 (66.66%)	AMP, SAM, FOX, CAZ, CRO, MEM, CN, TOB, CIP, LEV, SXT, ATM
44	***E. coli***	12 (66.66%)	AMP, SAM, FOX, CAZ, CRO, FEP, MEM, CIP, LEV, SXT, ATM, ETP
46	***E. coli***	8 (44.44 %)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, SXT
47	***E. coli***	10 (55.55%)	AMP, SAM, TZP,CN,TOB ,CIP, LEV, F, SXT, ETP
48	***K. pneumoniae***	7 (38.88%)	AMP, TZP, FEP, TOB, CIP, F, ETP
49	***E. coli***	15 (83.33%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, MEM ,AK, TOB ,CIP, LEV, SXT, ATM, ETP
51	***E. coli***	3 (16.66%)	AMP, SAM, MEM
54	***Raoultella planticola***	2 (11.11%)	AMP, FOX,
55	***Enterobacter cloacae***	1 (15.55%)	FOX
56	***K. pneumoniae***	7 (38.88%)	AMP, SAM, CRO, FEP, F, SXT, ATM
57	***E. coli***	3 (16.66%)	AMP, SAM, SXT
58	***E. coli***	4 (22.22%)	AMP, SAM, FOX, SXT
61	***E. coli***	7 (38.88%)	AMP, SAM, FOX, CRO, FEP, SXT, ATM
62	***E. coli***	8 (44.44 %)	AMP, SAM, FOX, CRO, FEP, CN, F, ATM
63	***E. coli***	4 (22.22%)	AMP, SAM, FOX, CIP
64	***E. coli***	4 (22.22%)	AMP, SAM, CN, CIP
65	***E. coli***	7 (38.88%)	AMP, TZP, CAZ, FEP, TOB, CIP, F
66	***Serratia liquefaciens***	5 (27.77%)	CN, TOB, CIP, SXT, ATM
67	***E. coli***	9 (50%)	AMP, CRO, FEP, CN, CIP, F, SXT, ATM, IPM
68	***E. coli***	2 (11.11%)	AMP, SXT
69	***K. pneumoniae***	9 (50%)	AMP, SAM, CRO, FEP, MEM, CN, CIP, F, ATM
70	***E. coli***	3 (16.66%)	AMP, TZP, FEP
72	***K. pneumoniae***	7 (38.88%)	AMP, SAM, CRO, FEP, F, SXT, ATM
73	***K. pneumoniae***	14 (77.77%)	AMP, SAM, CRO, FEP, MEM, AK, CN, TOB, F, SXT, ATM, ETP, IPM
74	***K. pneumoniae***	6 (33.33%)	AMP, SAM, CRO, FEP, SXT, ATM
76	***K. pneumoniae***	16 (88.88%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, MEM, AK, CN, TOB, CIP, LEV, F, ATM, ETP
77	***E. coli***	9 (50%)	AMP, SAM, CRO, FEP, AK, CN, TOB, CIP, ATM
78	***E. coli***	11 (61.11%)	AMP, SAM, CRO, FEP, MEM, TOB, CIP, SXT, ATM, ETP, IPM
79	***Serratia Plymuthica***	12 (66.66%)	CRO, FEP, MEM, AK, CN, TOB, CIP, F, SXT, ATM, ETP, IPM
80	***E. coli***	7 (38.88%)	AMP, SAM, FOX, CRO, FEP, CIP, ATM
81	***E. coli***	8 (44.44 %)	AMP, SAM, CRO, FEP, CN, CIP, SXT ,ATM
82	***E. coli***	1 (5.55%)	AMP
83	***E. coli***	0 (0%)	
84	***K. pneumoniae***	13 (72.22%)	AMP, SAM, CRO, FEP, MEM, AK, CN, TOB, CIP, F, SXT, ATM, IPM
85	***K. pneumoniae***	6 (33.33%)	AMP, SAM, CRO, FEP, SXT, ATM,

Code	Species	Resistant to (n)(%)	Antibiotics Symbols
86	***Enterobacter cloacae***	3 (16.66%)	CRO, F, ATM
87	***K. pneumoniae***	17 (94.44%)	AMP, SAM, TZP, FOX, CAZ, CRO, MEM, AK, CN, TOB, CIP, LEV, F, SXT, ATM, ETP, IPM
88	***E. coli***	14 (77.77%)	AMP, SAM, TZP, FOX, CAZ, CRO, MEM, CIP, LEV, SXT, ATM, ETP, IPM
89	***E. coli***	7 (38.88%)	AMP, SAM, TZP, CRO, FEP, CIP, ATM
90	***E. coli***	6 (33.33%)	AMP, SAM, TZP, CRO, MEM,CIP,
91	***E. coli***	11 (61.11%)	AMP, SAM,CRO ,FEP,MEM,TOB,CIP, SXT, ATM, ETP, IPM
92	***K. pneumoniae***	5 (27.77%)	AMP, SAM, CN, CIP, SXT
93	***E. coli***	7 (38.88%)	AMP, SAM, TZP, CAZ, FEP, SXT, ATM
94	***Citrobacter koseri***	0 (0%)	
96	***E. coli***	9 (50%)	AMP,SAM,CRO ,FEP,CN,TOB,CIP SXT,ATM
97	***E. coli***	7 (38.88%)	AMP,SAM, CAZ ,CRO ,FEP, SXT,ATM
98	***K. pneumoniae***	17 (94.44%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, MEM, AK, TOB, CIP, LEV, F, SXT, ATM, ETP, IPM
99	***K. pneumoniae***	17 (94.44%)	AMP, SAM, TZP, FOX, CRO, FEP, MEM, AK,CN,TOB, CIP, LEV, F, SXT, ATM, ETP, IPM

AMP: Ampicillin, SAM: Ampicillin/ Sulbactam, TZP: Piperacillin/ Tazobactam, FOX: Cefoxitin, CAZ: Ceftazidime, CRO: Ceftriaxon, FEP: Cefepime, MEM: Meropenem, AK: Amikacin, CN: Gentamicin, TOB: Tobramycin, CIP: Ciprofloxacin, F: Nitrofurantoin, SXT: Trimethoprim/ Sulfamethoxazole, ATM: Aztreonam, ETP: Ertapenem, IPM: Imipenem

Table 8: Antibiogram of *Genus: Pseudomonas& Sphingomonas* isolated from UTI infected patients (n=11) against 11 antibiotics.

Antibiotics names	Symbols	Sensitive (n)	Percentage (%)	Resistant (n)	Percentage (%)
Piperacillin/Tazobactam	TZP	6	54.54	5	45.45
Ceftazidime	CAZ	6	54.54	5	45.45
Cefepime	FEP	6	54.54	5	45.45
Meropenem	MEM	4	36.36	7	63.63
Amikacin	AK	8	72.72	3	27.27
Gentamicin	CN	5	45.45	6	54.54
Tobramycin	TOB	7	63.63	4	36.36
Ciprofloxacin	CIP	3	27.27	8	72.72
Levofloxacin	LEV	4	36.36	7	63.63
Aztreonam	ATM	6	54.54	5	45.45
Imipenem	IPM	8	72.72	3	27.27

Among the 100 isolates, there were 11 isolates of the genus: *Pseudomonas& Sphingomonas*, and there were 10 isolates of *Pseudomonas aeruginosa*, and they were numbered as follows 20 , 27, 34, 38, 39, 43, 50, 52, 53 and 100, and their resistance rates to the antibiotics applied to them were as follows respectively (45.45%), (54.54%), (45.45%), (81.81%), (18.8%), (9.09%), (72.72%), (54.54%), (54.54%) and (54.54%) while there was one isolate from *Sphingomonas paucimobilis*, and the percentage of its resistance to antibiotics was as follows (36.36%) .

Among one hundred isolates many isolates were MDR (72%) Figure (8) as they resistant against more than five to six or more antibiotics from different groups as table (9).

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Table 9: No. of resistant of isolates.

Sample cod	No. of Resist	Sample cod	No. of Resist	Sample cod	No. of Resist	Sample cod	No. of Resist
1	7	26	3	51	3	76	16
2	15	27	6	52	6	77	9
3	10	28	2	53	6	78	11
4	3	29	4	54	2	79	12
5	15	30	10	55	1	80	7
6	8	31	15	56	7	81	8
7	12	32	11	57	3	82	1
8	7	33	11	58	4	83	0
9	3	34	5	59		84	13
10	9	35	2	60		85	6
11	2	36	6	61	7	86	3
12	3	37	9	62	8	87	17
13	10	38	9	63	4	88	14
14	10	39	2	64	4	89	7
15	13	40	5	65	7	90	6
16	10	41	7	66	5	91	11
17	14	42	12	67	9	92	5
18	4	43	1	68	2	93	7
19	5	44	12	69	9	94	0
20	5	45	10	70	3	95	4
21	5	46	8	71		96	9
22	3	47	10	72	7	97	7
23	9	48	7	73	14	98	17
24	5	49	15	74	6	99	17
25	2	50	8	75	3	100	6

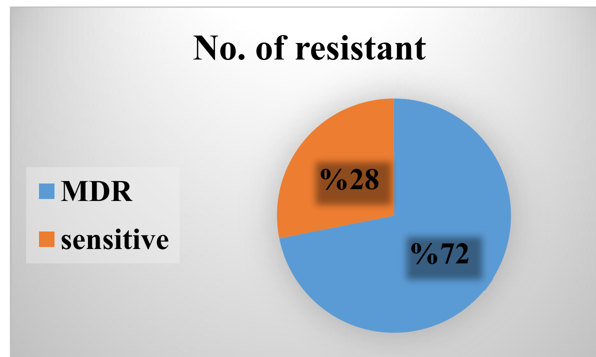

Fig. 8: Frequency of No. of resistant isolates.

After conducting statistical operations, the samples were selected are (2,21,38,45 and 87) as the most resistant isolates to antibiotics to conduct genetic and protein experiments on them Table (10).

Table 10: The most resistant isolates.

Sample code.	Species	Resistant to (n)(%)	Antibiotics Symbols
2	***E. coli***	15 (83.33%)	AMP, SAM, TZP, FOX, CAZ, CRO, FEP, MEM ,AK, TOB ,CIP, LEV, SXT, ATM, ETP
21	***E. faecalis***	5(71.42%)	E,RD,CIP, LZD,TE
38	***P. aeruginosa***	9 (81.81%)	CAZ, FEP, MEM, CN, TOB, CIP, LEV, ATM, IPM
45	***A. baumannii***	10(83.33%)	SAM, TZP, CAZ, CRO, FEP, MEM,CN,TOB,CIP,LEV
87	***K. pneumonia***	17 (94.44%)	AMP, SAM, TZP, FOX, CAZ, CRO, MEM, AK, CN, TOB, CIP, LEV, F, SXT, ATM, ETP, IPM

Genomic identification of MDR isolates:

The different five extensive MDR bacterial isolates were identified through 16S rDNA sequencing. They identified as *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Enterococcus faecalis* and *Klebsiella pneumoniae*

4. Discussion

In our current study of the prevalence of UTI in Egyptian patients due to bacterial infections; One hundred mid-stream urine samples were collected from 71 females and 29 males, who were attending the outpatient departments (OPDs) (64%) and in-patient departments of Suez Canal Authority hospital during over 8 months' period (may 2020 – march 2021). All had clinical evidence of urinary tract infection, diagnosed by urologists. The age of patients included in the study ranged from 10 to ≥ 60 years with average age 58-year. Patients on antibiotic therapy were excluded from the study. The total of recovered microbial isolates included gram negative bacteria (88 isolates), gram positive ones (only 8 isolates) and 4 yeast isolates. The gram negative isolates included 70 isolates (80%) lactose fermenting, while 17 isolates (20%) where lactose non-fermenting. Two isolates were recovered on blood agar with a beta hemolysis, in addition to 22 (65%) isolates of gram-negative with beta hemolysis and 10(29%) isolates of gram-negative alpha hemolysis.

Almost our findings came parallel to previous reports of (Flores-Mireles *et al.*, 2015b) who confirmed the incorporation of a wide range of pathogens including both gram negative and gram positive bacteria and yeasts as a causative gents of Urinary tract infections (UTIs) in males and female patients. Also, our_investigation noticed the development of some complicated symptoms especially among older ages of men and women who were using medical caster during some surgical operations, which agrees with previous reports of (Flores-Mireles *et al.*, 2015b)and other reports. (Cheng *et al.*, 2016; Saraswathy and Ramalingam, 2011; Vranakis *et al.*, 2014)Standing on Vitek report of biochemical analysis of one hundred isolates; isolates belonging to different 5 genera and species; Out of one hundred isolates two isolates were defined as fungal isolates belonging to *Candida species* which represent 2 *species Candida tropicalis* and only one was *Candida dubliniensis. In* addition to one hundred isolates belonging to different genera. These included 18 bacterial isolates their genera and species with ratios were clarified as Gram-negative Bacteria which contain *E. coli* with ratio 46(44%), *Klebsiella pneumoniae* 24(25%), *Pseudomonas aeruginosa* 10(10%), *Enterobacter cloacae* 2(2%) and *Acinetobacter baumannii, Citrobacter koseri, Serratia liquefaciens, Serratia Plymuthica, Sphingomonas paucimobilis, Morganella morganii* with 1(1%) for each of them and Gram-positive bacteria clarified as follows, *Staphylococcus lentus* and *Staphylococcus haemolyticus* with ratio 2(2%) for each them , and *Staphylococcus xylosus, Enterococcus faecalis, Staphylococcus saprophyticus* and *Enterococcus spp* with 1 % for each of them , and four isolates of Yeast watch divided in to *Candida tropicalis* 3(3%) and *Candida dubliniensis* 1(1%) .

Our findings came in agreement with findings of many authors, (Flores-Mireles *et al.*, 2015b)who reported the in order of prevalence, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus*

(GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida* spp. For complicated UTIs, the other causative agents are in order of prevalence,

Enterococcus spp., *K. pneumoniae*, *Candida* spp., *S. aureus, P. mirabilis*, *P. aeruginosa* and GBS The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC)

Also, mostly there is a high percentage of agreement with other authors including (Butel, 2007; Singh *et al.*, 2019; Kaur and Kaur, 2021) who confirmed the isolation of *Klebsiella*, *Proteus, Pseudomonas* and *Enterobacter* as a main cause of urinary tract infection in males and females. In addition to (Butel, 2007)who reported The microbial etiology of UTIs is deemed to be well established and frequent pathogens like *E. coli* and *S. saprophyticus* are associated with population acquired acute uncomplicated infection. Meanwhile *Klebsiella, Enterococcus*, *Proteus* Species, *Enterobacter, Bacillus, Shigella* are known to confer uncomplicated cystitis and phylonephritis , were reported by ; Singh *et al.*(2019).

The most prevalent isolate in this investigation was *E. coli* 46(44%), followed by *K. pneumoniae.* 24(25%), then *P. aeruginosa,* 10(10%) *while* the rest of isolates were slightly reported in all cases of

investigated patients *E. faecalis* 2(2%) and *, A. baumannii,* 1(1%) these results came in agreement with some authors including (Bennett *et al.*, 2014), who reported *E.coli* as the most prevalent isolate, (Walsh and Collyns, 2017b) reported *K. pneumoniae.*ac the most prevalent pathogen in urinary tract infection in males and females , (Flores-Mireles *et al.*, 2015b; Thomas and Tolley, 2008)reported *Proteus vulgaris*. *P. mirabilis* as the main cause of uncomplicated urinary tract infection while the pathogens *Klebsiella*, *Pseudomonas*, *Staphylococcus* and *Corynebacterium urealyticum* were also reported as incorporated in the UTI in both males and females. In addition (Reyes *et al.*, 2006; Walsh and Collyns, 2017b) reported the incorporation of some enteric bacteria in exerting the uncomplicated symptoms of urinary tract infection. (Bennett *et al.*, 2014; Flores-Mireles *et al.*, 2015b) reported *Pseudomonas. Aeruginosa* as an important cause of the uncomplicated urinary tract infection.

Among one hundred of recovered isolates in our trial the majority were resistant to most antibiotics (MDR) bacterial pathogens (72%) as they were resistant against more than five to six or more antibiotics from different groups. On comparing our results with other those of other investigators (Walsh and Collyns, 2017b)reported *S. aurous*: as has being described as the ‘prince of pathogens’ due to its array of virulence factors and success in colonizing, and causing infections, in humans including its resistance to majority of antibiotics. In addition, *S. saprophyticus* was also reported second only to *E. coli* in causing uncomplicated infections in sexually active, young women; however, it rarely causes infections in men or older women. It has a unique adhesion protein, UafA, which facilitates its adhesion to uroepithelial cells. It produces urease, as well as having various transport proteins, which enable it to survive, and multiply, in the face of osmotic and pH changes according to reports of (Bennett *et al.*, 2014; Flores-Mireles *et al.*, 2015b)Also, UTIs due to this organism have been found to be more common in late summer and autumn; and may be concomitant with vaginal candidiasis (Bennett *et al.*, 2014).

However, *Staphylococcus epidermidis*. was reported as being of very low virulence in an otherwise healthy host, is well adapted to adhering to, and forming biofilm on, foreign material _ such as a urinary catheter. It can produce autolysins which bind directly to plastic and other compounds; and bacterial products such as accumulation associated protein, Bap homologue protein, polysaccharide intercellular adhesion and extracellular DNA contribute to biofilm formation (Bennett *et al.*, 2014).

Also, parallel to our findings, the main two species involved in infections are *Enterococcus faecalis* and *Enterococcus faecium*, the latter being intrinsically resistant to penicillins such as ampicillin or piperacillin. Enterococci are well-adapted to colonizing the human gastrointestinal tract and receipt of antibiotics by the host, such as a cephalosporin, can alter the balance in favour of *enterococcal* proliferation as they are naturally tolerant to various antimicrobial classes. *Enterococci* can produce various adhesins and aggregation substances which establish and maintain biofilms (Bennett *et al.*, 2014; Flores-Mireles *et al.*, 2015a). *Enterococci* are of relatively low pathogenicity and may colonize catheters rather than causing symptomatic infection. Similarly, their presence may prevent a more pathogenic species from successfully invading the urinary tract e i.e. treatment of an asymptomatic enterococcal bacteriuria can be followed by a symptomatic infection, due to a different organism (Walsh and Collyns, 2017a).

Concerning the Multidrug resistance (MDR) of the recovered bacterial isolates, during the last few decades, the incidence of microbial infections has increased dramatically. Continuous development of antimicrobial drugs in treating infections has led to the emergence of resistance among the various strains of microorganisms. Multidrug resistance (MDR) is defined as insensitivity or resistance of a microorganism to the administered antimicrobial medicines despite their earlier sensitivity to it (Méndez-Vilas, 2013). According to WHO, 2020, these resistant microorganisms are able to combat attack by antimicrobial drugs, which leads to ineffective treatment resulting in persistence and spreading of infections. Studies from WHO 2020 , report have shown very high rates of resistance in bacteria such as *Escherichia coli* against antibiotics as cephalosporin and fluoroquinolones, *Klebsiella pneumoniae* against cephalosporin and carbapenems, *Staphylococcus aureus* against methicillin, *Streptococcus pneumonia* against penicillin, Nontyphoidal *Salmonella* against fluoroquinolones, *Shigella* species against fluoroquinolones, *Neisseria gonorrhoeae* against cephalosporin, and *Mycobacterium tuberculosis* against rifampicin, isoniazid, and fluoroquinolone causing common infections (Nikaido, 2009; Organization, 2014) (like urinary tract infections, pneumonia, and bloodstream infections) and high percentage of hospital-acquired infections. In addition, Antimicrobial resistance is associated with high mortality rates and high medical costs and has a significant impact on the effectiveness of antimicrobial agents (Tanwar *et al.*, 2014).

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