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Distribution of Antibacterial Resistance among Pathogenic Bacterial Isolates from Patient in Al-Shatrah General Hospital

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Abstract

Nowadays antimicrobial resistance is a huge phenomenon worldwide. The current article aims to detect the most antibiotic resistant among different kinds of antibiotics that extensively use in Iraq, to reduce the antibiotic resistance phenomenon, detect the most effective antibiotic can eliminate or kill pathogenic isolates. Two hundred clinical specimens were collected from patients who are hospitalized Al-Shatrah General Hospital. All samples were directly inoculated in enrichment media after had been obtained from urine, blood, ear swabs, sputum, wound swabs and diagnosed depending on the biochemical tests and molecular investigation that were done. The isolated pathogens were examined against 35 kinds of antibiotic including 13 Beta-lactams, 4 Aminoglycosides, 4 Fluoroquinolones, 3 Macrolides, 2 Glycopeptides, 2 Polymyxins, and one for each of Tetracycline, Chloramphenicol, Nitrofurantoin, Linezolid, Tazobactam, and Bacitracin using Kirby disc diffusion method. The results classified isolated bacteria into 60 (40.8%) isolates Gram-positive and 87 (59.2%) were Gram-negative isolates. While 69 (47%) bacterial isolates were isolated from males; 78 (53%) bacterial isolates were isolated from females, the most common isolated pathogens were *Staphylococcus* sp. 40 (27%), *Streptococcus* sp. 20 (13.6%), *Proteus* sp. 24 (16%), *Escherichia coli* 29 (19.7%); whereas, the least isolated bacteria is *Salmonella* typhi 2 (1.4%). While, the highest isolates were 41 isolates (36%) within age group (36-45); the least isolates were 19 isolates (12.9%) within age group (56-65). Bacitracin and Oxacillin were resisted by 10 bacterial species in this study. Also, the results found Levofloxacin, Ceftriaxone, Cefotaxime, Ciprofloxacin, Meropenem, Tazobactam, Imipenem are more effective in compare to other antibiotics.

Key words: Staphylococcus, Streptococcus, Pseudomonas, Antibiotic resistance, MDR and Amoxicillin.

Introduction

The phenomenon of antimicrobial resistance (AMR) presents a significant peril to both human health and well-being, while also exerting a detrimental impact on global economies. The anticipated annual losses resulting from antimicrobial resistance in the United States vary from 21,000 to 34,000 million dollars, while in Europe, these losses amount to approximately 1500 million Euros (EU, 2010). Antibiotics are pharmaceutical agents utilized to combat bacterial illnesses. Utilizing them is likely to result in life-saving outcomes. Antibiotic resistance continues to pose an escalating challenge. This phenomenon occurs when bacteria undergo genetic mutations that enable them to develop resistance against the effects of an antibiotic (Alwatar, *et al*., 2023). Antibiotics are naturally occurring chemical compounds primarily derived from fungi, which, when employed appropriately, provide the potential to preserve human lives. Improper utilization of antibiotics can lead to the development of resistance (Sydnor, and Perl, 2011). The ability of these microorganisms to transmit and proliferate, resulting in illnesses that are resistant to specific antibiotics, presents a significant contemporary issue (David, 2019). The issue of AMR poses a significant challenge to the successful prevention and treatment of diseases caused by many microorganisms, including bacteria, parasites, viruses, and fungi (WHO, 2001). The concept of antimicrobial resistance resulting from excessive antibiotic usage was anticipated by Alexander Fleming, who posited that there may be a future scenario where penicillin becomes readily available to the general public through commercial channels. Furthermore, there exists a potential risk wherein individuals lacking knowledge may inadvertently administer an insufficient dosage, thereby exposing their microorganisms to nonlethal levels of the medication, thereby fostering resistance (Smith,

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2001; Amábile-Cuevas, 2007). In the absence of the development of novel and more potent antibiotics, there exists a tangible potential for a future in which common infections and small injuries can result in fatalities, and where intricate medical interventions like surgery and chemotherapy become excessively hazardous (Fleming, 2018). The phenomenon of AR poses a significant global danger, as it has the potential to result in widespread outbreaks of illness if appropriate preventive measures are not implemented. In contemporary society, the prevalence of antibiotic resistance has resulted in extended hospitalization periods, escalated healthcare expenditures, and heightened mortality rates (Golkar *et al.*, 2014). A strong positive link has been observed between AR and the inappropriate use of antibiotics (Goossens, 2009; Apisarnthanarak and Mundy, 2008). The urine samples obtained from patients diagnosed with urolithiasis in Tikrit city, Iraq, had a prevalence rate of 45% for UTI, among these bacteria 48% were classified as gram-negative, while 16% were classified as gram-positive. Furthermore, a significant proportion of the isolates that were identified were classified within the Enterobacteriaceae family (Al-Jebouri, *et al.*, 2013). A range of strategies were employed to inhibit bacterial proliferation, including the utilization of natural products, bacterial metabolic compounds (Sabah, and Kadim, 2024), physical approaches such as direct and indirect electric currents (Abdulhusein, *et al.*, 2024), and the application of metal complexes (Ali, *et al.*, 2020). This study aimed to isolate, identify pathogenic bacteria from patients attended AL-Shatrah general Hospital, detect the most antibiotic resistant among different kinds of antibiotic that extensively use in Iraq, to minimize the abuse of the antibiotics, to reduce the antibiotic resistance phenomenon, detect the most effective antibiotic to eliminate or kill pathogenic isolates.

I. Materials and Methods

2.1. Sampling

In a period ranging from February 1, 2023, to March 31, 2024, a total of two hundred samples were collected from patients who were admitted to AL-Shatrah General Hospital. The specimens included in this study consist of 35 samples of urine, 16 samples of blood, 15 samples of nasal swab, 22 samples of wound swab, 23 samples of sputum, 32 samples of ear swab, 27 samples of vaginal swab, and 30 samples of stool swab. Employing conventional microbiological methods, all samples were inoculated both aerobically and non-aerobically in enrichment media (Blood agar, Nutrient agar, and Chocolate agar) and incubated overnight at a temperature of 35℃. Subsequently, the Gram stain was conducted to classify our isolates into gram-positive and gram-negative categories (MacFaddin, 2000; Abdulhusein and Kadim, 2024). The collected samples were classified into six distinct groups according to the age distribution of the patients, including 5-15 years, 16-25 years, 26-35 years, 36-45 years, 46-55 years, and 56-65 years. Furthermore, the samples were categorized, based on their gender, into males and females.

2.2. Biochemical Tests and Analytical Profile Index (API 20) strip method

All grown bacterial colonies on the mentioned above media were sub-cultured by using differential and specific media including (MacConkey agar, Mannitol salt agar, *Salmonella-Shigella* agar, Urea Agar, Urea broth, and Cetrimide agar). The IMVIC tests were carried out by following the standard procedure. The bacterial growth was introduced into test tubes with peptone water and thereafter cultured at 35℃ for 24h. Following this, a mixture of two drops of kovag's reagent was applied, and the observation of a red or pink hue was deemed indicative of a positive result for the presence of tryptophanase activity. The methyl red test entails the introduction of bacterial growth into glucose phosphate broth, followed by incubation at 35℃ for 48 hours. Following the incubation period, 5 drops of methyl red are introduced, and the visualization of a red coloration signifies a positive outcome. The Voges-Proskauer test was performed using the identical method, but employing 0.2 ml of 40% KOH and alpha-naphthol as the reagent. The outcome yielded a noticeable crimson hue within a span of one hour. Overnight, the bacterial inoculum was cultivated in Simmon's citrate agar at 35℃. The experiment yielded a positive outcome, as seen by the agar's color transition to a profound blue shade (Microbe Online, 2014; Abdulhusein and Kadim, 2024). To investigate the hemolytic properties of the isolated species, they were cultured on blood agar that had been previously prepared by incorporating 5% human blood agar base (Barrow, and Feltham, 1993; Abdulhusein and Kadim, 2024). Subsequently, all petri plates were incubated at 35℃ for between 24 and 48 hours. The results were determined by observing the presence of transparent zones surrounding the developing colonies. The isolates were categorized according to the specific type of

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hemolysis found in nearby areas of the former colonies following the designated incubation period. The analysis of colony morphology was conducted using blood agar. Following incubation at 37°C for 24 hours, numerous characteristics of the colonies were observed and quantified. The characteristics examined in this study included the morphology, pigmentation, colony size, and the magnitude of hemolysis detected on blood agar (Seddiq *et al*., 2023). An extensive examination was conducted to explore the structure and coloration of bacterial colonies. The study encompassed the examination of the shape and pigmentation of colonies on petri plates following their subsequent cultivation and overnight incubation at 37°C (Sánchez-Porro *et al*., 2011).

2.3. Biochemical test

2.3.1. Oxidase test

As part of the experimental protocol, the introduction of tetra-dimethyl-para-phenylenediamine dihydrochloride into the filter paper was thereafter succeeded by the application of bacterial colonies onto the aforementioned filter paper, the findings were documented as the manifestation of a blue-violet hue within a temporal interval of 20 seconds (Harley and Prescott, 2002; Abdulhusein *et al*., 2023).

2.3.2. Catalase

Colonies cultivated on the growth medium were subjected to the application of two drops of hydrogen peroxide (H_2O_2) with a concentration of 3%. It was observed that the colonies displaying a positive reaction generated gas bubbles, whilst the negative isolates did not manifest any bubble formation (Harley and Prescott, 2002; Abdulhusein *et al.*, 2023).

2.3.3. Coagulase test

To assess the presence of coagulase, a test tube containing rabbit plasma was injected with bacterial growth and incubated overnight at a temperature of 37°C. The development of a clot was deemed an appositive result, while the absence of a clot was interpreted as a negative result (Barrow, and Feltham, 1993).

2.3.4. Nitrate Reduction

The nitrite broth and agar tubes were inoculated with freshly cultured 24 hours of bacterial growth. Subsequently, the tubes were incubated at a temperature of 35°C for a duration ranging from 24 to 48 hours. The results were interpreted by observing the alteration in color and gas production.

2.4. Bacitracin susceptibility and PYR test

Furthermore, the bacitracin test is employed for the purpose of differentiating *S. pyogenes* from other β-hemolytic streptococci that exhibit PYR positivity, including *S. iniae* and *Streptococcus porcinus*. In order to conduct a bacitracin susceptibility test, it is imperative to establish a subculture of the strain under investigation on a sheep blood agar plate (SBA). Otherwise, the placement of the bacitracin disk on a main plate may yield inconsistent outcomes. The strain under investigation is streaked with multiple individual colonies of a pure culture on an SBA agar plate, followed by the placement of a disk containing 0.04 U of bacitracin directly onto the SBA plate. A zone of inhibition encircling the disk, observed following an overnight incubation at 35°C in a 5% CO2 environment, serves as an indicator of the strain's susceptibility (Spellerberg and Brandt, 2016). The PYR test is a commonly employed colorimetric technique that utilizes quick colorimetry to differentiate *S. pyogenes* from other β-hemolytic streptococci that exhibit similar morphology, such as *Streptococcus dysgalactiae* subsp. *equismilis*. Additionally, this test is utilized to detect the presence of the enzyme pyrrolidonyl aminopeptidase. This enzyme catalyzes the hydrolysis of L-pyrrolidonyl-β-naphthylamide (PYR) into β-naphthylamide, resulting in the formation of a red hue upon the addition of a cinnamaldehyde reagent. A rapid assay can be conducted on paper strips containing desiccated chromogenic substrates for the pyrrolidonyl aminopeptidase enzyme (Spellerberg and Brandt, 2016).

2.5. Assessment the Antibiotic sensitivity testing (AST)

The Kirby-Bauer disc diffusion technique was employed to conduct the AST, following the specifications outlined in the 2016 Clinical and Laboratory Standards Institute (CLSI) recommendations. Amoxicillin/ Clavulanic Acid (AMC- 10µg), Penicillin G (PG- 10 µg, Oxacillin (OX- 1µg), Ampicillin (AMP-10µg), Ticarcillin (TIC-75µg), Imipenem (IPM-10µg), Mezlocillin (MEZ- 75µg), Aztreonam (ATM-30µg), Ceftriaxone (CRO-30µg), Ceftazidime (CAZ-30µg), Cefalotin (CF-30µg), Cefazolin (CZ-30µg), Cefotaxime (CTM-30µg), and Meropenem (MEM-10µg), Amikacin (AMK- 30µg), Gentamicin (GEN-10µg), Neomycin (NEO- 30µg), Kanamycin (KAN-30 µg), Levofloxacin (LEV-5µg),

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Ciprofloxacin (CIP-5µg), Ofloxacin (OFX-5µg), Norfloxacin (NOR-10µg), Erythromycin (ERY-15µg), Clindamycin (CLI-2µg), Lincomycin (LIN-2µg), Vancomycin (VAN-30µg), Teicoplanin (TEC-30µg), Colistin (COL-10µg), Polymyxin B (PB-300 U), Tetracycline (TET-30µg), Chloramphenicol (CHL-30µg), Nitrofurantoin (NIT-300µg), Linezolid (LZD-30µg), Tazobactam (TZB-4µg), and Bacitracin (BAC-10U) directly applied against our isolates using Kirby-Bauer disc diffusion technique and the results were interpreted accordingly (CLSI, 2016). The McFarland Standards were employed to establish a standard procedure for quantifying the estimated bacterial population in a liquid suspension. This objective was accomplished through a comparative analysis of the visibility or turbidity levels observed in the test suspension and the McFarland Standard. The 0.5 McFarland Standards set by clinical microbiology laboratories serve as the established benchmark for conducting antibiotic susceptibility testing and evaluating the performance of culture medium. To achieve a concentration of 1×10^8 CFU/mL, the turbidity of all suspensions containing pathogenic bacteria was corrected following the guidelines outlined in McFarland Standard No 0.5. This adjustment was made using a sterile saline solution (Abdulhusein and Kadim, 2024).

2.6. Molecular Verification

The molecular identification was done by using conventional PCR assay. Bacterial chromosomal DNA was extracted and purified using respectively QIAamp and QIAquick kits (Germany). Depending on method mentioned by Wilson, (2001) primers were designed and used in order to amplify the bacterial genes, table (1). A 50 µL total volume of PCR mixture was used during the PCR reaction composed of [200 µM dNTP, 0.5 µM primers, 2.5 U Taq polymerase, 100ng template DNA, 10 mM Tris-HCl, 1.5 mM MgCl2, and 50 mM KCl], the program for the PCR reaction was 3 min denaturation at 94°C, 60 sec. annealing at 45°C, 60 sec. extension at 72°C, and Final extension for 7 min at 72°C. The results were tested using electrophoresis (Mihdhir *et al.*, 2016).

Table 1. Primers used in current investigation.

Bacteria	Primers	Ref.
S. aureus	F-(GTAGGTGGCAAGCGTTACC) R-(CGCACATCA GCGTCAG)	Al-Musawi *et al*., 2014
S. epidermidis	F-(GTAGGTGGCAAGCGTTACC) R-(CGCACATCA GCGTCAG)	Al-Musawi *et al*., 2014
S. saprophyticus	F-(GAAGTCGTAACAAGG) R-(CAAGGCATCCACCGT).	Couto *et al*., 2001
S. pyogenes	F-(AAGAGAGACTAACGCATGTTAGTAAT) R-(ATTTTCCACTCCCACCATCA)	Kulkarni *et al*., 2016
S. pneumonia	F-(TGGCTCAGGACGAACGCTGGC) R-(CGGCTGCTGGCACGTAGTTAGC)	Bishop *et al*., 2009
P. aeroginosa	F-(GGGGGATCTTCGGACCTCA) R-(TCCTTAGAGTGCC CACCCG)	Spilker *et al*., 2004
E. coli	F-(GACCTCGGTTTAGTTCACAGA) R- (CACACGCTGACGCTGA CCA)	Mamun *et al*., 2016
S .marcesence	F-(GAGTTTGATCCTGGCTCAG) R-(AGAAAGGAGGTGATCCAGCC)	Yoon and Park, 1998
K. pneumonia	F-(ATGACGTCAAGTCATCATGG) R- (AGGAGGTGATCCAGCCGCA)	Klausegger *et al*., 1999
Citrobacter sp.	F-(TCTGAGAGGATGACCAGCCA) R-(GGGACTTAACCCAACATTTC)	Jacoby *et al.*, 2011
Salmonella typhi	F-(GGAACTGAGACACGGTCCAG) R-(CCAGGTAAGGTTCTTCGCGT)	Kaabi *et al*., 2019
Proteus sp.	F-(GGAAACGGTGGCTAATACCGCATAAT) R-(GGAAACGGTGGCTAATACCGCATAAT)	Adnan *et al*., 2014

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III. RESULTS AND DISCUSSION

The samples were classified according to the gender into 108 (54%) males and 92 (46%) females, among these categorize the results found that while 69 (47%) bacterial isolates were isolated from males; 78 (53%) bacterial isolates were isolated from females. Also the samples were classified based on their sources into 62 samples from Urinary tract infection disease unit, 38 samples pulmonary patients and Respiratory disorders, 30 samples from Gastroenteritis patients, 16 samples from patients with Endocarditis, 22 samples from wound infection and burns patients, and 32 samples Otitis media. The result revealed that 40, 30, 27, 24, 21, and 4 bacterial species were respectively isolated from UTI patient's, Pulmonary disorder and Respiratory disorders patient's, Otitis media, Gastroenteritis, wound infection and burns, and Endocarditis, table (2).

Table 2: Distribution of Samples based on the gender and disease

	Disease						Total
	UTI	Pulmonary disorder and Respiratory disorders	Gastroenteritis	Endocarditis	wound infection and burns	Otitis media	
Number of samples	62	38	30	16	22	32	200
Male	27	23	17	9	13	19	108
Female	35	15	13	7	9	13	92
Isolated bacteria	40	30	24	5	21	27	147

Previous investigation elucidated that of 128 samples were collected during period 5/ 2019 to 10/2019 in Iraq the most isolated bacteria is *S. aureus* (19.5%), followed by *E .coli* (11.7%), and the lowest isolated bacteria *S. viridians* and *k. pneumonia* (Alwatar *et al*., 2023), also in this study the total number of females samples were higher than males samples. The collected samples were classified into six distinct groups according to the age distribution of the patients, and the samples were randomly collected including 31 samples within 5-15 years, 33 samples within 16-25 years, 38 samples within 26-35 years, 41 samples within 36-45 years, 32 samples within 46-55 years, and 25 samples within 56-65 years. The results of current study discovered that the isolated species were 22 isolates (14.9%), 24 isolates (16.3%), 27 isolates (18.3%), 32 isolates (21.7%), 23 isolates (15.6%), and 19 isolates (12.9%), respectively distributed within age groups (5-15), (16-25), (26-35), (36-45), (46-55), and (56-65). Table (3).

Table 3: Distribution of samples and isolated pathogenic species based on age groups

Age groups	No. of samples	%	No. of Isolates	%
5-15	31	15.5	22	14.9
16-25	33	16.5	24	16.3
26-35	38	19	27	18.3
36-45	41	20.5	32	21.7
46-55	32	16	23	15.6
56-66	25	12.5	19	12.9
Total	200	100	147	100

Erlear investigation in Mosul/ Iraq clarified that of 174 samples were obtained the males samples were 135 more than females 38 and the most common bacteria is *S. aureus* (48.2%), and the highest incidence was found in individual aged (20-49); whereas the lowest rate of infection was found in individual aged more than 50 years (Aiber *et al.*, 2022). The results of this work revealed that that isolated bacteria were (73.5%) 147 of two hundreds specimens. The findings are classified the isolated bacteria into two groups 60 (40.8%) isolates were Gram-positive and 87 (59.2%) were Gram-negative isolates. According to the

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biochemical tests, enzymatic activities, and the API 20 tests 17% (25 isolates) *Staphylococcus aureus*, 6.12% (9 isolates) *Staphylococcus epidermidis*, 4.7% (7 isolates) *Streptococcus pneumonia*, 3.4% (5 isolates) *Streptococcus pyogens*, 5.44% (8 isolates) *Streptococcus viridians* and 4% (6 isolates) *Staphylococcus saprophyticus*. Whereas the gram negative bacteria, which were isolated distributed between Enterobacteriaceae and Pseudomonadaceae including 4.7% (7 isolates) *Klebsiella pneumonia*, 9.5% (14 isolates) *Pseudomonas aureginosa*, 19.7% (29 isolates) *Escherichia coli*, 0.7% (1 isolates) *Citrobacter freundii*, 5.44% (8 isolates) *Serratia marcescens*, 10.2% (15 isolates)10.2% *Proteus mirabilis*, (2 isolates) 1.4% *Salmonella typhi*, (9 isolates) 6.12%*Proteus vulgaris*, and (2 isolates) 1.4% *Citrobacter koseri*, table (4). Figure (1).

Table 4: Bacterial species and number of isolates isolated from patients

No	Bacterial species	Number of isolates	%	
1	*Staphylococcus aureus*	25	17	40 (27%)
2	*Staphylococcus epidermidis*	9	6.12	
3	*Staphylococcus saprophyticus*	6	4	
4	*Streptococcus pneumonia*	7	4.7	20 (13.6%)
5	*Streptococcus pyogens*	5	3.4	
6	*Streptococcus viridians*	8	5.44	
7	*Klebsiella pneumonia*	7	4.7%	
8	*Pseudomonas aureginosa*	14	9.5%	
9	*Escherichia coli*	29	19.7%	
10	*Citrobacter freundii*	1	0.7	3 (2%)
11	*Citrobacter koseri*	2	1.4	
12	*Serratia marcescens*	8	5.44	
13	*Proteus mirabilis*	15	10.2	24 (16%)
14	*Proteus vulgaris*	9	6.12	
15	*Salmonella typhi*	2	1.4	
Total		147	100	

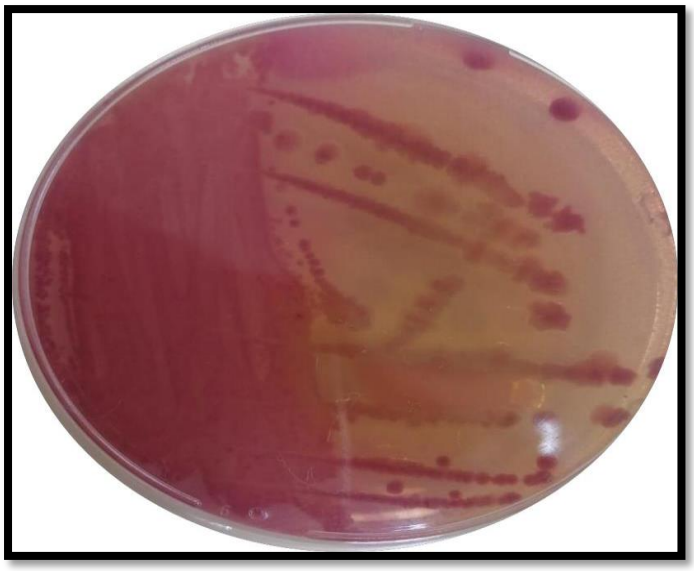

Figure 1. *Escherichia coli* on MacConkey agar.

During the current study 35 kinds of antibiotic disks were used and categorized into 13 antibiotic kinds belonging to Beta-lactams [Amoxicillin/Clavulanic Acid (Penicillin G, Oxacillin, Ampicillin,

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Ticarcillin, Imipenem, Mezlocillin, Aztreonam, Ceftriaxone, Ceftazidime, Cefalotin, Cefazolin, Cefotaxime, and Meropenem], 4 antibiotic types belonging to Aminoglycosides [Amikacin, Gentamicin, Neomycin, and Kanamycin], 4 antibiotics within Fluoroquinolones [Levofloxacin, Ciprofloxacin, Ofloxacin, and Norfloxacin], Macrolides and Lincosamides [Erythromycin, Clindamycin, and Lincomycin], 2 Glycopeptides [Vancomycin, and Teicoplanin], Polymyxins [Colistin, and Polymyxin B], Tetracycline, Chloramphenicol, Nitrofurantoin, Linezolid, Tazobactam, and Bacitracin were tested against *S. aureus, S. epidermidis, S. pneumonia, S. pyogens, S. viridians, S. saprophyticus, K. pneumonia, P. aureginosa, E. coli, C. freundii, S. marcescens, P. mirabilis, S. typhi, P. vulgaris,* and *C. koseri* and the results were varied from one antibiotic to another. The results found that *P. aureginosa* showed resistance to AMC-10µg, PG-10µg, Ceftazidime, Cefalotin, Cefazolin, Oxacillin, Ampicillin, Vancomycin, Lincomycin, Bacitracin, Clindamycin, and Nitrofurantoin, table (5), and figure (2). Bacitracin and Oxacillin were resisted by 10 bacterial species in this study. Also, the results of our investigation found that Levofloxacin, Ceftriaxone, Cefotaxime, Ciprofloxacin, Meropenem, Tazobactam, Imipenem are more effective against the tested isolates in compare to other types of antibiotics. Moreover, oflaxacilin and Amixacin showed high activity against all tested isolates.

Table 5. The Bacterial sensitivity test

No	Antibiotics	Bacterial species														
		S. aureus	*S. epidermidis*	*S. pneumonia*	*S. pyogens*	*S. viridians*	*S. saprophyticus*	*K. pneumonia*	*P. aureginosa*	*E. coli*	*C. freundii*	*S. marcescens*	*P. mirabilis*	*S. typhi*	*P. vulgaris*	*C. koseri*
1	AMC- 10µg	22	19	26	30	20	23	22	0	12	22	18	27	19	15	12
2	Chloramphenicol	30	33	32	21	19	19	0	11	29	28	19	19	21	33	17
3	PG- 10µg	41	29	39	33	35	0	0	0	0	0	0	25	0	7	0
4	levofloxacin	28	24	32	29	27	30	15	19	30	26	24	23	26	24	33
5	Ceftriaxone	29	31	41	29	36	25	13	22	31	37	33	41	27	43	30
6	CAZ-30µg	31	25	33	15	29	24	19	0	31	27	25	21	23	24	27
7	Cefalotin	41	42	39	34	30	21	0	0	18	13	0	29	20	15	14
8	Cefazolin	39	40	41	29	19	24	9	0	26	21	0	25	19	11	19
9	Cefotaxime	30	35	42	30	34	27	16	17	35	39	35	45	28	45	29
10	Ciprofloxacin	24	28	33	29	32	30	28	17	31	26	25	25	27	26	31
11	Meropenem	31	29	35	26	34	30	33	22	34	28	26	29	24	33	34
12	Tazobactam	33	35	39	29	22	22	19	23	25	33	30	33	26	43	27
13	Amikacin	21	29	8	5	12	25	21	23	22	25	23	23	18	29	27
14	Oxacillin	31	39	31	22	7	0	0	0	0	0	0	0	0	0	0
15	Ampicillin	39	31	42	29	34	0	0	0	20	15	0	29	21	17	9
16	Aztreonam	0	0	10	15	7	21	9	23	33	43	35	43	19	45	25
17	Tetracycline	31	11	29	27	25	23	0	12	27	29	11	11	17	19	18
18	Ticarcillin	40	35	39	33	8	17	0	25	30	35	29	37	24	33	22
19	Mezlocillin	37	29	41	29	6	24	0	13	26	25	25	33	27	35	25
20	Imipenem	53	54	47	30	29	28	29	29	29	35	27	33	23	33	29
21	Gentamycin	23	31	6	16	12	22	0	21	21	25	21	21	18	25	23
22	Vancomycin	20	22	21	15	19	29	0	0	7	0	0	0	0	0	0
23	Colistin	0	7	0	0	0	0	11	13	14	13	15	0	0	0	10
24	Lincomycin	31	39	26	17	19	16	0	0	0	0	0	0	0	9	0
25	Bacitracin	21	17	23	21	14	0	0	0	0	0	0	0	0	0	0
26	Clindamycin	39	39	31	23	27	25	0	0	0	0	0	0	0	9	0
27	Norfloxacin	26	35	14	11	0	30	33	9	37	39	29	41	28	43	28
28	Erythromycin	31	39	25	25	28	0	9	10	9	9	9	0	0	7	0
29	Linezolid	31	40	33	21	26	23	0	0	0	0	0	11	0	15	0

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30	Teicoplanin	18	19	17	11	23	26	0	0	0	0	0	9	0	0	0
31	Ofloxacin	28	30	16	15	29	28	29	21	31	39	29	39	25	41	30
32	Nitrofurantoin	25	31	29	19	6	31	19	0	23	23	5	7	0	11	21
33	Polymyxin B	10	13	0	0	0	0	13	15	25	15	15	0	0	8	12
34	Neomycin	21	31	0	0	17	21	11	15	18	19	0	0	17	23	16
35	Kanamycin	23	29	0	9	24	19	0	11	19	37	23	23	20	25	22

Our result were similar to the results obtained by Aiber *et al.*, in (2022), who found that the most isolated species were sensitive to ciprofloxacin, nitrofurantoin, and amikacin; also *P. aeroginosa* showed resistant, levofloxacin, and cholramfenicol exhibited highly activity against *E. coli*.

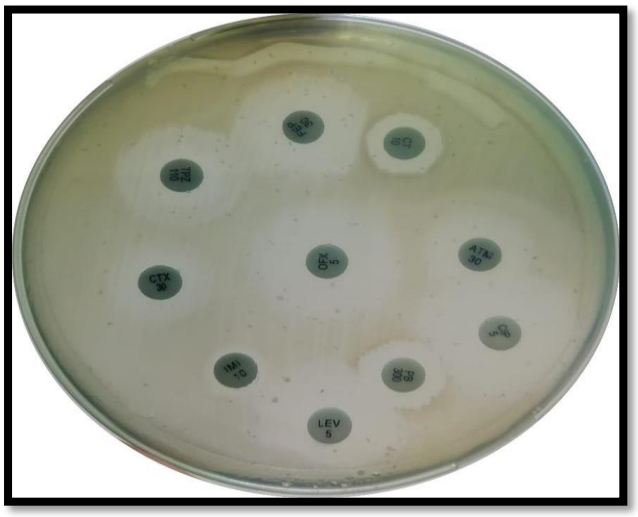

Figure 2. The Antibiotic sensitivity test of *Pseudomonas aeroginosa*.

The samples were classified according to the gender into 108 (54%) males and 92 (46%) females, among these categorize the results found that while 69 (47%) bacterial isolates were isolated from males; 78 (53%) bacterial isolates were isolated from females. Also the samples were classified based on their sources into 62 samples from Urinary tract infection disease unit, 38 samples pulmonary patients and Respiratory disorders, 30 samples from Gastroenteritis patients, 16 samples from patients with Endocarditis, 22 samples from wound infection and burns patients, and 32 samples Otitis media. The result revealed that 40, 30, 27, 24, 21, and 4 bacterial species were respectively isolated from UTI patient's, Pulmonary disorder and Respiratory disorders patient's, Otitis media, Gastroenteritis, wound infection and burns, and Endocarditis, table (2).

V. CONCLUSSION

All isolated bacterial species are opportunistic pathogens that cause UTIs, Pulmonary disorder and Respiratory disorders, Otitis media, Gastroenteritis, wound infection and burns, and Endocarditis. While of the patients in this research were males; 78 (53%) bacterial isolates were isolated from females and 69 (47%) bacterial isolates were isolated from males. *P. aeroginosa* displayed resistant to 10 antibiotics in clinical isolates (MDR). Medications of the Oxacillin, Colistin, Vancomycin, Linezolid, Teicoplanin, Polymyxin B, Neomycin, and Kanamycin exhibited high resistance, while the Cefpirome , Ceftazidime/Avibactam , Ceftolozane/Tazobactam, Faropenem, Doripenem, Latamoxef and Tigecyline were most efficient against tested isolates. Levofloxacin, Ceftriaxone, Cefotaxime, Ciprofloxacin, Meropenem, Tazobactam, Imipenem are more effective against the tested isolates in compare to other types of antibiotics. Moreover, oflaxacilin and Amixacin showed high activity against all tested isolates. The highest rate of infection was found in individual aged (36-45); whereas the lowest rate of infection

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was found in individual aged (56-65) from all the 147 isolates. The results classified isolated bacteria into 60 (40.8%) isolates Gram-positive and 87 (59.2%) were Gram-negative isolates. the most common isolated pathogens were *Staphylococcus* sp. 27%; whereas, the lowest isolated bacteria is *Salmonella* typhi 1.4%.

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