Study of Some Bacteriological Parameter on Pseudomonas Aeruginosa Isolated from Patients with UTI

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Abstract: Background: Urinary tract infections (UTIs) are a serious health problem that affects millions of people each year. Urinary tract infections are the second most common type of infection in the body. One of the causes of infection in the urinary tract is caused by microorganisms such as Pseudomonas aeruginosa. The pathogenic potential was proved by their frequent isolation from clinical samples and association with diseases. from various clinical samples and perform antimicrobial susceptibility testing (AST) by Vitek.

Methodology: This study was conducted to investigate the prevalence of antibiotic resistance among Pseudomonas aeruginosa bacteria isolated from the urine of patients Suffering from UTI in Karbala governorate.

(100) isolated samples of Pseudomonas aeruginosa were isolated from the urine of UTI patients with ages ranging from 5 to 65 years for both sexes and the samples were diagnosed based on a number of biochemical tests and sensitivity test using VITEK 2 system technology.

Results: In this study, it was found that 100 Pseudomonas aeruginosa bacterial isolate (75% female and 25% male) of patients with urinary tract infection, Pseudomonas aeruginosa showed a high resistance to antibiotics. The study also showed that the percentage of adult females with urinary tract infection due to Pseudomonas aeruginosa is higher than males, reaching 75%.

The most effective antimicrobial against Pseudomonas aeruginosa were (colistin sulphate and gentamicin). While a high resistance rate towards the antibiotics (Ceftazidime , ciprofloxacin , levofloxacin , amikacin , Tetracycline , Imipenem , Meropenem , Trimethoprim ,

Piperacillin).

Conclusion: It was concluded that Pseudomonas aeruginosa, which was increase resistant rate to antibiotics and this is observed through this study against antipseudomonal drugs indicates the need to develop targeted approaches to help control antimicrobial resistance.

Key words: Urinary tract infections; Pseudomonas aeruginosa; Virulence factors; VITEK 2

1. Introduction

1.1. Urinary tract infections

infections of the urethra, bladder, ureters, or the kidneys, which comprise the urinary tract. *Escherichia coli* bacteria and *Pseudomonas aeruginosa* **cause the majority of UTIs**, but many other bacteria, fungi, and parasites may also cause UTIs [1].

Females have a higher risk for UTIs than most males, probably because of their anatomy; other risk factors for UTIs include any condition that may impede urine flow (e.g., enlarged prostate, congenital urinary tract abnormalities, and inflammation). Patients with catheters or those who undergo urinary surgery and men with enlarged prostates are at higher risk for UTIs. Symptoms and signs of UTI vary somewhat depending on sex, age, and the area of the urinary tract that is infected; some unique symptoms develop depending on the infecting agent[1,2].

UTIs are diagnosed usually by isolating and identifying the urinary pathogen from the patient; there are some home tests available for presumptive diagnosis. There are home remedies for UTI, but most may, at best, help reduce the risk or discomfort of UTIs. They are not considered cures for the disease. There can be many complications of urinary tract infections, including dehydration, sepsis, kidney failure, and death [3,4].

If treated early and adequately, the prognosis is good for most patients with a UTI. Although there is no vaccine available for UTIs, there are many ways a person may reduce the chance of getting a UTI. Urinary Tract Infection UTI is a bacterial infection affecting urinary tract. When bacteria from the rectal area enter the urinary tract via the urethra to the bladder and multiply in the urine, an infection occurs. In many cases bacteria first travel to the urethra. When bacteria multiply an infection can occur. An infection limited to the urethra is called urethritis. If bacteria move to the bladder and multiply, a bladder infection called cystitis. If the infection is not treated promptly, bacteria may then travel further up the ureters to multiply and infect the kidneys, called pyelonephritis [5].

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span [1,2]. UTIs account for more than 8 million visits to physician's offices, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually [3,4]. UTIs are the second most common infection of any organ system and the most common urological disease in the United States, with a total annual cost of more than $3.5 billion [5].

These infections are more common in females than in men. Incidence in women in the age of 20—40 years ranges from 25 to 30% whereas in older women above 60 years of age it ranges from 4 to 43% [6,7,8]. UTIs can be classified as uncomplicated or complicated [9,10].

The recognized predisposing factors in complicated UTIs are anatomic defects, vesico uretic reflux (VUR), obstruction, surgery, metabolic diseases like diabetes mellitus and generalized immunosuppression especially in patients of organ transplant [11,12,13,14,15,16].

Catheterization of urinary tract is one of the most common factor which predisposes the host to complicated UTIs [17,18,19,20]. Instillation of catheter may lead to damage of mucosal layer, which disrupts the natural barrier and allows bacterial colonization [21]. Organisms can gain entry via extraluminal route [22] by moving across the outer lumen of catheter or by intraluminal route by directly entering the interior of catheter [23].

The organisms most commonly responsible for catheter-associated UTIs are Escherichia coli, Proteus mirabilis, **Pseudomonas aeruginosa**, *Klebsiella pneumoniae* and *Streptococcus faecalis* [6,24,25,26]. In case of *E. coli*, the epidemiological, experimental and clinical studies have established the role of multiple virulence factors of *E. coli* like adhesins operative through type-I fimbriae and P fimbriae, O serotypes, K1 capsule, serum resistance, hemolysins, cytotoxic nectrotizing factor (CNF) and siderophores (enterochelin and aerobactin) in relation to uncomplicated and complicated UTIs [2,27].

However, there is paucity of literature in relation to pathogenesis of UTIs caused by *P. aeruginosa*. Despite advances in antimicrobial therapy, the mortality and morbidity associated with P. aeruginosa induced UTIs remain significantly high. This unfavorable outcome is due to our inability to develop therapeutic strategies to prevent the disease which in turn is due to incomplete understanding about the pathogenesis of the disease [27].

1.2. Virulence factors of uropathogenic *P. aeruginosa*

P. aeruginosa is the third most virulent is It is multifactorial and is attributed to factors related to cells such as genes and lipopolysaccharides (LPS), whip, sticky and non-adhesive lashes As well as with exogenous enzymes or secretory virulence Factors such as protease, elastase, phovo lipase, pyocyanin, exotoxin A, exoenzyme S, hemolysin (rhamnolipids) and Siderophores [28,29,30,31].

These Factors have been shown to play an important role Role in the pathogenesis of *P.aeruginosa* induced disease Infections such as respiratory infections, burns Wound infections and keratitis [32-36]. However, Limited reports are available on the role of these Virulence traits in urinary tract infections , burn wound infections and keratitis In addition to determining virulence factors,*P. aeruginosa* tends to form biofilms which are the most important factors of bacterial virulence. [32,33,34,35,36].

1.3. Antibiotics resistant by bacteria

Bacterial organisms are now resistant to many routinely used antibiotics causing treatment failures. The emergence of resistance as a major problem has drawn attention to a need for better diagnostic techniques. Vitek 2 is one such instrument which is fully automated system, designed to decrease the turnaround time for the identification of bacteria and determination of antimicrobial susceptibilities. The instrument also provides a more hands-off approach than the original Vitek instrument t[37].

The WHO has declared carbapenem resistant *P. aeruginosa* as a major and critical public health issue in need of developing therapeutic intervention [36].The current new agents/combinations such as ceftolozane/tazobactam and ceftazidime/avibactam have increased the options with which the opportunistic pathogen is treated [37] However, both treatments have already encountered in vitro and in vivo resistance [38,39].

Despite the emergence of potential new treatments in phase III clinical trials such as meropenem - vaborbactam, mipenemrelebactam murepavadin and cefepime-zidebactam, the efficacy will not be assessed until prolonged period of consistent clinical use [40-41]. Thus, the current last resort for UTI treatment is colistin [42].

2. Material and methods

2.1. Isolation of *P.aeruginosa* bacteria

100 bacterial isolate of *P. aeruginosa* were obtained from urine sample obtained from patients with UTI of both sexes for age groups (5-65),

2.2. Sample culturing

After collecting urine samples from urinary tract patients, and within a period not exceeding an hour, they were transferred to the microbiology laboratory and planted on culture media with blood agar, MacConkey agar and Nutrient agar .It was incubated at 37 °C for 24 hours to ensure its purity and to obtain single colonies , as shown in the picture (1).

Picture (1) of *P.aeruginosa* colonies on Nutrient agar

2.3. Diagnosis of *P.aeruginosa*

The bacteria were initially diagnosed based on isolation were identified based on several biochemical and microbiology tests and were used (VIETK-). 2 COMPACT system) for a more accurate diagnosis. It is considered one of the best devices for identifying types of microorganisms within a short period and in a serious manner.

The device is developed by the French company Biomerieux. It automatically determines the type of bacteria in the device by performing 64 Biochemical tests . Without the need to perform any other tests and to recognize the negative and positive bacteria of the dye Cram and yeast and viruses in addition to checking sensitivity to antibiotics. The most important thing that distinguishes it is that it identifies living cells only using the identification card, where the level of diagnosis of the object is determined through a map of its tests and compared with the classification characteristics of the device, giving the organism a probability ratio and level of confidence [42] .

biochemical tests As shown in the table (1) .

Table (1) Results of biochemical tests for isolates of *P.aeruginosa*

N	Test	Result
1	Blood Agar	+ (β- hemolysis)
2	Gram stained Test	_
3	Oxidase Test	+

4	Catalase Tests	+
5	Motility Test	+

Negative test: (-)

Positive test: (+)

2.4. Determination of antibiotic sensitivity

To obtain and recognize the sensitivity of *P. aeruginosa* to antibiotics, a diagnostic and sensitivity test for the bacterial isolates was performed using the advanced VITEK-2 device technology.

2.5.Statistical Analysis

Qi square was used in data analysis and results were considered statistically significant at P≥0.05 .

3. Results and Discussion

3.1 prevalence of pseudomonas aeruginosa according to age

The results showed that after collecting (100 urine samples) from urological patients admitted to Imam Al-Hijjah Hospital during the period from the first of January to the end of May 2021, Among the 100 urine samples, *P. aeruginosa* colony growth was estimated in (100) male and female patients with age range from (15-65) years old as shown in Table No (3-1).

Table (3-1) Bacteria *P.aeruginosa* according to age among collecting samples

N	Age categories	*P.aeruginosa*	Male samples	Female samples	Number
1	25-15	24	6	18	24
2	35-25	36	5	31	36
3	45-35	12	2	10	12
4	55-45	18	8	10	18
5	65-55	10	4	6	10
		100	25	75	100

3.2 prevalence of pseudomonas aeruginosa according to gender

This study included 100 urine sample of patients infected with pseudomonas infection 75 was female and 25 was male as show in table (3.2).

Table (3-2) *P.aeruginosa* according to gender among collecting samples

Patient with pseudomonas	No.	Total
Male	25	100
Female	75	

3.3 Antimicrobial susceptibility test of *P.aeruginosa* isolated from UTI patients.

A study on antimicrobial resistance patterns previously conducted in this study **,** the results of improved by using the Vitec2 system technology that the bacteria are highly resistant to antibiotics as shown in Table (3)and figure (3.1)

Table (3.3) Bacterial *P.aeruginosa* antibiotic resistance

N	Antibiotics	Class of antibiotics	R	S	I
1	Ceftazidime	Cephalosporin	90	1	9
2	Ciprofloxacin	Cephalosporin	91	4	5
3	Levofloxacin	Fluoroquinlones	100	-	-

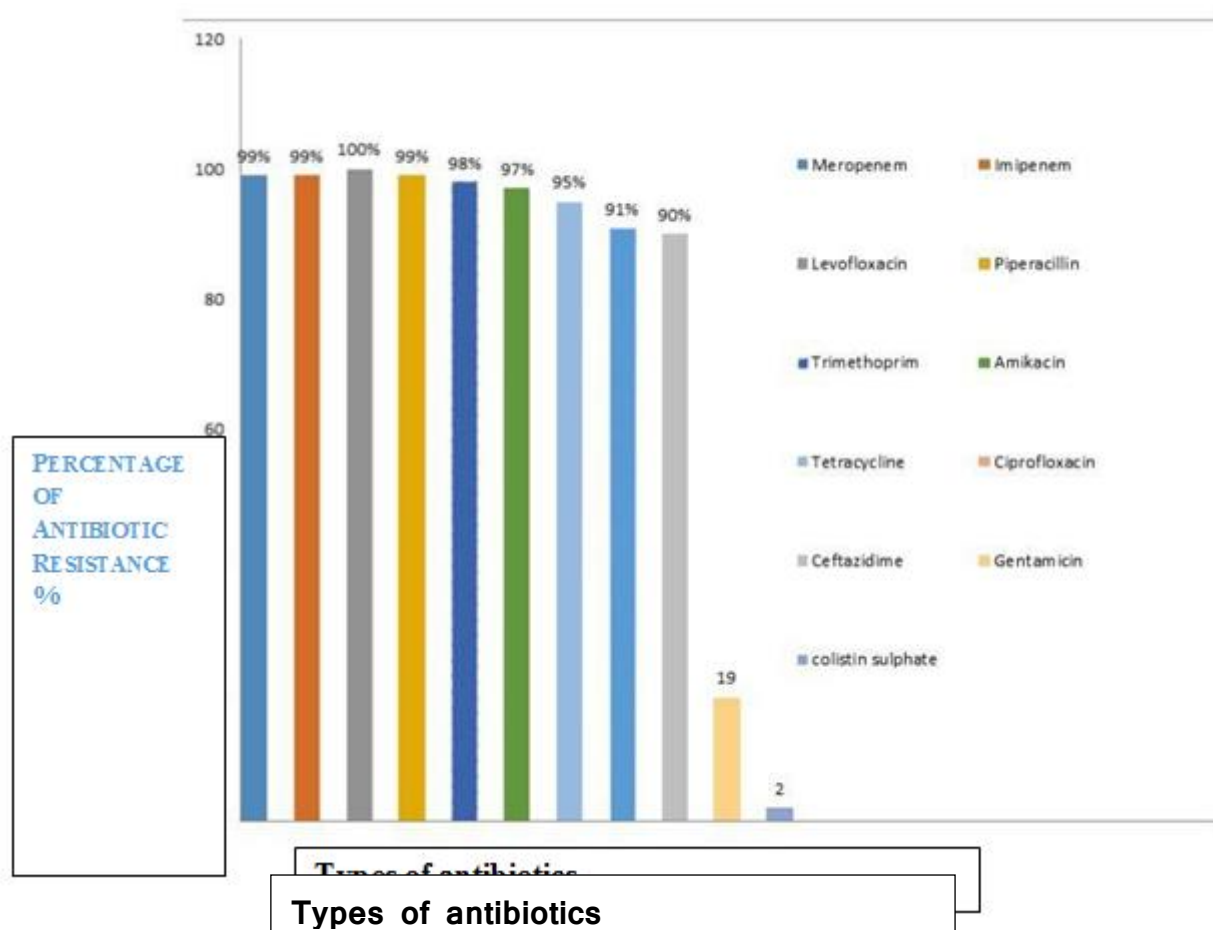|					
4	Amikacin	Aminoglycoside	97	2	1
5	Tetracycline	Tetracyline	95	3	2
6	Imipenem	Carbapenem	99	1	-
7	Meropenem	Carbapenem	99	-	1
8	Trimethoprim	Sulfonamides	98	-	2
9	Piperacillin	Pencillin	99	-	1
10	colistin sulphate	Polymyxin	2	98	-
11	Gentamicin	Aminoglycoside	19	81	-

Figure 3.1 the percentage of antibiotic resistance of *pseudomonas aeruginosa* isolated from patients with UTI.

The current study was similar to other study, found that *P. aeruginosa* has a strong tendency to become a multidrug-resistant pathogen. The susceptibility significantly declined after 2007, particularly for carbapenem, ceftazidime and ciprofloxacin [44].

For the purpose of our study, we chose the Gram negative bacterium *P.aeruginosa* because of its medical importance and innate ability to develop antibiotic resistance.

In our study, this bacterium was shown to be more susceptible to colistin sulphate (98%) and gentamycin (81%). as shown in Fig.(3.1)

By contrast, the isolates tested in this study showed increased resistance to antibiotics in the following categories:

(i) Fluoroquinlones (levofloxacin **69%);**

(ii) Pencillin (Piperacillin**99%);**

(iii) Carbapenem (Meropenem **,** Imipenem, **99%);**

(iv) Sulfonamides (Trimethoprim **53%);**

(v) Aminoglycoside (amikacin98%)**;**

(vi) Tetracyline(Tetracyline **95%);**

(vii) Cephalosporin(ciprofloxacin 91% **,** Ceftazidime90%). In contrast to previous reports.[45] Resistance values for carbapenems oscillated between 25 and 33% for meropenem and imipenem, respectively. In the United States, the National Health care Safety Net work reported that *Pseudomonas aeruginosa* is the sixth most common nosocomial pathogen. While.[46] reported 9.7% resistance rate to at least one aminoglycoside and 19.3% to at least one carbapenem. Other study[47] ported resistance

Rate for cephalosporins was 10.3% (cefepimeorceftazidime) compared to 6%, 12% and53% for cefepime, ceftazidime and cefotaxime, respectively and resistance for one fluoroquinolones was reported in 21.6% of isolates. Overall, the MDR was reported to be 14.2% (previous study), much lower than the 100% observed in the present study.

The difference between the two studies could be explained by the presence of intrinsic genetic differences between circulating isolates, which is corroborated by the presence of various resistance mechanisms and /or genes that contribute to virulence and resistance to various drugs representatives of eight antibiotic classes.

4. Conclusion

Pseudomonas aeruginosa, which was increase resistant rate to antibiotics, the high resistance rate that had been observed through this study against antipseudomonal drugs indicates the need to develop targeted approaches to help control antimicrobial resistance.

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