ANTIBIOTIC RESISTANCE PROFILES OF PSEUDOMONAS AERUGINOSA ISOLATED FROM CLINICAL SAMPLES

1. INTRODUCTION

Pseudomonas aeruginosa, a gram-negative bacteria and is the most commonly connected Pseudomonas strain with human illnesses (Streeter and Katouli 2016).

Those are responsible for cystic fibrosis patients’ persistent lung infections, in particular ventilator-associated pneumonia, wound infections in severe burn patients, septicemia, and catheter-associated (UTLs), multi kinds of infectious diseases have been identified linked to P. aeruginosa, all of which have complicated etiologies (Streeter and Katouli 2016).

As a result, Pseudomonas aeruginosa is identified as an opportunistic pathogen since it predominantly creates nosocomial infections in immune compromised people; total of 20 samples of P. aeruginosa obtained from a variety of locations in Baghdad hospitals isolated from: burns, wounds and sputum collected from October 2021 to the April 2022, isolates cultured by Using culture conditions, biochemical assays, and the VITEK-2 compact system. For all bacterial isolates, the antibiotic sensitivity test is valid according to the minimum inhibitory concentration (MICs). The maximum level of resistance was demonstrated to be against Tigecycline (8), followed by Cefazolin (64), while high sensitive was to Ciprofloxacin (0.25), followed by Levofloxacin (0.5) and Gentamicin (1), cefepime (1), Ceftazidime (2) and Imipenem (2), Amikacin (2) and Piperacillin /Tazobactum (8) respectively according to MICs results of each antibiotics.

Key words: Antibiotic Resistance; Pseudomonas aeruginosa; Polysaccharit; Tigecycline.
which serve as a key role in antimicrobial resistance and in the formation of mature biofilms. (Laverty et al. 2014).

Virulence factors are particular to mature biofilms production and antibiotic resistance including: (flagellum, type IV pili, extracellular virulence factors, siderophores, hydrogen cyanide, pyocyanin, lipopolysaccharide (LPS) O-antigen, and Quorum Sensing Systems (QS), lipase, elastases, exotoxin A [ETA], protease IV, exoenzymes S, T and Y, rhamnolipids, exotoxin U, phospholipase C, alginate) (Wu et al. 2015).

Antimicrobial resistance obtained by chromosomally encoded systems’ expression and/or functions are modified by mutational events, or by acquiring resistance genes on plasmids (Kadum et al. 2019).

According to that, in the current study we aimed to: The Determination of Antibiotic Resistance in Pseudomonas aeruginosa: (β-lactam, fluoroquinolones, cephalosporins, glycylicycline, carbapenems and aminoglycosides antibiotics), to investigate if there's a class of synthetic wide-spectrum antibiotics that kill bacteria by inhibiting cell wall, cell membrane, protein synthesis or DNA synthesis.

2. MATERIALS AND METHODS

2.1. Ethics statement

This research was performed in accordance with the principles of the Declaration of Helsinki. The study was conducted according to the institutional review board (IRB) standards for research and ethics approval of the relevant institutional review boards (Department of Biology, and College of Science at the University of Çankiri, (2021). the clinical information for patients with wounds, burns, sputum was provided by Baghdad hospitals, For this type of isolates, written informed consent was obtained from hospitals and laboratory directors.

The research study was performed in accordance with the Declaration of hospitals Baghdad/Iraq guidelines.

2.2. Bacterial isolation and characterization

All specimens were cultivated on MacConkey agar (Riedel et al. 2022, Sarmad et al., 2022), and incubated the plates aerobically for 24 hours at 37°C. The pure colony of bacteria was then chosen to undergo 64 biochemical tests, including oxidase, catalase, lactose, and additional, as well as confirmation of the isolates' identification using the VITEK2 compact system and device-specific diagnostic kits for P.aeruginosa.

2.3. Bacterial diagnosis by Compact Vitek 2 system (bioMérieux, France):

Vitek 2 system is one of the modern diagnostic systems device It is used in diagnosing bacterial isolates at the species level, and it can also determine the sensitivity of the bacteria to antibiotics, and it works by having special cards containing 64 very small holes, each hole designated for a specific test. It is a large-sized device equipped with two chambers, the first of which is known as the filling chamber, as the holes in the card are filled with bacterial suspension. And the second is the incubation chamber, in which the model is incubated to the second day, after which the result is read (Marko et al. 2012, Mohammed et al., 2021)

followed the steps as per the manufacturer's instructions: Wound, burn and sputum swabs were implanted on the culture media (MacConkey's agar) has been set samples are incubated at a temperature (37 celsius) for a period of (24) hours, during which growth was observed on these growth media, and Dilution was made with a special tube for the manufacturer containing (3) milliliters of distilled water supplied by the company, and a little growth were transferred to it by the standard conveyor.

The suspension's turbidity was compared to that of a conventional fixed turbidity solution (McFarland) with the turbidity meter supplied by the company which is called the DensiCHEK Plus™, because the ultimate concentration within the tube must be in the range of (0.65-0.5), We repeat the dilution process when measurement result of the sample
appears with an inappropriate concentration of the actual percentage specified for turbidity to obtain the correct percentage, the tubes were placed in their bearing after adding to each tube a special test strip based on the diagnosis of gram-negative, since gram-negative bacteria have their own strip (GN cassette)).

The code number affixed to the bearing and to the test tape was entered into the computer attached to the device, through the (smart carrier) accessory device, in order for the device to recognize the bearing and tapes, after that entering the data of the sample, including giving the sample number, the name of the patient, and the source of the sample (burns–wounds–(sputum) respiratory system), and transferring the bearing containing the tubes and their tapes (GN cassette) to the system, then placing it first in the (Filler field), which automatically fills the tapes with bacterial suspension, and once the procedure is finished, the device gives a (transfer instruction).

The bearing was transferred to the second field, (the reader), who first cut the tapes (GN cassette) and gave a Burden in the form of a digital signal, as it kept the tapes (GN cassette). As for the bearer containing the tubes, it exited the device, the tapes were left for (24 hours) at (37°C) temperature, then I read the data of each sample, which is saved on the computer attached to the vitek-2 compact system.

Incubation and card sealing: Around 35.5+1.0°C, all kinds of card were incubated and each cards was picked once each 15 minutes from the incubators, , these reactions readings were transmitted into the optics system, So its incubators returned till other moment of reading; These data were gathered at interval of 15 minutes all through the incubating phase.

Reactions to testing: Raw data calculation are carried out and comparing for every testing to evaluate the reaction. Testing outcomes show as ‘+’, ‘-‘, ‘(-)’ or ‘(+)' of the VITEK 2 Compact.

2.4. Antibiotics cards
Antibiotics used in the study are given in Table 1

<table>
<thead>
<tr>
<th>Antibiotic group</th>
<th>Antimicrobial agents</th>
<th>CODE</th>
<th>CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins 1\textsuperscript{st} generation</td>
<td>Cefazolin</td>
<td>CZ</td>
<td>4, 16, 64</td>
</tr>
<tr>
<td>Glycylcyclines (Tetracyclines)</td>
<td>Tigecycline</td>
<td>TGC</td>
<td>0.75, 2, 4</td>
</tr>
<tr>
<td>Beta lactam/beta lactamase inhibitor combination</td>
<td>Piperacillin /Tazobactum</td>
<td>TZP</td>
<td>2/4, 8/4, 24/4, 32/4, 32/8, 48/8</td>
</tr>
<tr>
<td>Carapenems</td>
<td>Imipenem</td>
<td>IPM</td>
<td>1, 2, 6, 12</td>
</tr>
<tr>
<td>Cephalosprins 4\textsuperscript{th} generation</td>
<td>Cefepime</td>
<td>FEP</td>
<td>2, 8, 16, 32</td>
</tr>
<tr>
<td>Cephalosprins 3\textsuperscript{rd} generation</td>
<td>Ceftazidime</td>
<td>CAZ</td>
<td>1, 2, 8, 32</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>Amikacin</td>
<td>AMK</td>
<td>8, 16, 64</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GM</td>
<td>4, 16, 32</td>
<td></td>
</tr>
<tr>
<td>Flouroquinolones 3\textsuperscript{rd} generation</td>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>0.5, 2, 4</td>
</tr>
<tr>
<td>Flouroquinolones 2\textsuperscript{nd} generation</td>
<td>Levofloxacin</td>
<td>LEV</td>
<td>0.25, 0.5, 2, 8</td>
</tr>
</tbody>
</table>

2.5. Antibiotic test susceptibility
The manufacturer’s guidelines were followed while utilizing Vitek 2 compact system to assess antibiotic susceptibility in strains isolated of *Pseudomonas aeruginosa* using software version 8.1 and AST-GN76 cards, all of these isolates were examined, and a standard strain (Pseudo ATCC\textsuperscript{®} 27853TM) was utilized as a control.

The AST-GN76 cards provide a set of 19 antibiotics for assessing drug susceptibility.
this study the profile of antibiotics with various concentrations were employed to determine the sensitivity of Gram-negative bacteria: Cefazolin (4, 16, 64), Tigecycline (0.75, 2, 4), Piperacillin PI /Tazobactum (2/4, 8/4, 24/4, 32/4, 32/8, 48/8), Amikacin (8, 16, 64), Ceftazidime (1, 2, 8, 32), Imipenem (1, 2, 6, 12), Cefepime (2, 8, 16, 32), Gentamicin (4, 16, 32), Levofloxacin (0.25, 0.5, 2, 8), Ciprofloxacin (0.5, 2, 4) as shown in picture.

The broth micro dilution technique, as specified by the Clinical and Laboratory Standards Institute (CLSI), was used to estimate the minimum inhibitory concentrations (MICs) of antibiotics (Patel et al. 2015).

2.5.1. Vitek 2 compact system for determining the resistance and sensitivity of the bacteria to antibiotics

The AST-GN76 cards were used to evaluate and examine the susceptibility of clinically important Gram-negative bacteria.

Dilution was made with a special tube for the manufacturer containing (2.8) microliters of distilled water supplied by the company, and a little growth were transferred to it by the standard conveyor.

The suspension's turbidity was compared to that of a conventional fixed turbidity solution (McFarland) with the turbidity meter supplied by the company which is called the DensiCHEK Plus™, because the ultimate concentration within the tube must be in the range of (0.65-0.5). We repeat the dilution process when measurement result of the sample appears with an inappropriate concentration of the actual percentage specified for turbidity to obtain the correct percentage, and following the manufacturer's instructions, the cards (AST-GN76 cards) were completely placed, locked, and subjected to a kinetic fluorescence measurement, and using integrated vacuum equipment, Manufacturing-quality microorganisms were put into identity cards as well as pure strains of bacterial isolate preparations. The bacterial suspension was inserted in a test tube and on a designated rack (cassette), while the AST-GN76 cards were inserted into the correct suspension tube, and the transfer tube was fitted into a neighboring slot, Immediately in the compact reader-incubator module of the VITEK 2, the loaded cassette was inserted. The bacterial suspension was injected in small-channels that covered every test well when the vacuum was eliminated and the air was reintroduced in the port, and infected cards were automatically sealed before being placed on the carousel incubator, and the whole card were automatically incubated at 35.5°C every 15 minutes, every card was lifted first from incubator carousel and entered into the optical system before being placed back in the incubator until the next read.

During the incubation phase, data was gathered at 15-minute intervals, and final identification findings were recorded and achieved in less than 24 hours. All of the used cards were immediately discarded in a rubbish can.

3. RESULTS AND DISCUSSION

3.1. Bacterial Isolation and Identification

Standard identification procedures used to be able to recognize twenty (20%) P. aeruginosa isolates from all clinical samples, including colony characteristics on MacConky agar, the result was brownish green coloration after incubation at 37°C for an overnight period and irregular feathered edges, grape-like odor (amino acetophenone) and approximately 2 mm in diameter and lactose non fermenting (14). The findings of biochemical tests show negative reactions for methyl red, lactose and Indol, and positive reactions for catalase, gelatin hydrolysis, citrate utilization, and oxidase, the capability of VITEK®2 in recognizing P. aeruginosa is (99-98 %) (It indicates excellent). (Riedel et al. 2022).

Each card in the data base is based on the positivity of biological processes, with VITEK2 compact system confirmation of all isolates.
3.2. Evaluation the Susceptibility of Antibiotics Among Isolates

The results of bacterial resistance isolates to antibiotics showed in Table 4.7 due to the VITEK 2 Compact system with AST-GN76 cards, that showed the highest resistance was against Tigecycline (8) the result similar to (Kim et al. 2018, Keyal et al. 2020), followed by Cefazolin (64), result was close to (Ali et al. 2020) and show high sensitive to Ciprofloxacin (0.25) the same result as (Abdallah and Gabur 2021) and (Faiz et al. 2022), followed by Levofloxacin (0.5) the result close to (Ali et al. 2020) and Gentamicin (1), Cefepime range (1), Ceftazidime (2) and Imipenem (2) respectively, the results relatively the same as (Abdallah and Gabur 2021, Faiz et al. 2022), Amikacin (2) and Piperacillin/Tazobactum (8) the result was similar to (Faiz et al. 2022) depend on MICs outcomes of each antibiotics. (Table 4.7).

Cefazolin is belong to cephalosporins group and Cephalosporins is a Cell wall synthesis inhibitors (Prabhurajeshwar 2019), and consider an MDR P. aeruginosa infection due to previous hospitalization, prior antibiotic use and Involvement in an intensive care unit (ICU) are all risk factors for increasing resistance to this antibiotic (cefazolin) (Ding et al. 2021).

According to a study from India, over half of Pseudomonas aeruginosa bacteria are resistant to cephalosporin medicines. (Molla et al. 2020)

Furthermore, a comprehensive evaluation of antibiotic resistance in Bangladesh published in 2019 indicated that most infections had significant levels of resistance and that typical first-line antimicrobial medicines were ineffective in most cases (Ahmed et al. 2019).

The results also indicates the resistance to tigecycline and that approved by (Cui et al. 2021) which explain the role of mutation in the molecular mechanism through a newly found high-level tigecycline resistance mechanism mediated by plasmids Tet (X), and according to (Riedel et al. 2022). It has poor antimicrobial action against P. aeruginosa.

When such incidents happen (spontaneous mutations in essential chromosomal genes, transfer of entire new genes or groups of genes from another species), the end result can range from minor changes in microbial sensitivity that can be addressed with higher dosages of the medicine to total loss of sensitivity. Typically, spontaneous random mutations in bacterial populations cause chromosomal drug resistance.

The possibility of such a mutation being helpful is limited, and the potential of giving drug resistance is even rarer. Despite this, such changes do occur due to the enormous number of microorganisms in each population and the constant process of mutation (Kathleen 2017).

Due to the findings of our study, fluoroquinolones demonstrate susceptibility. This is due to their involvement in binding to DNA gyrase and a similar enzyme, and this mechanism of action assures that fluoroquinolones provide broad-spectrum efficacy. Ciprofloxacin and levofloxacin are two most frequently prescribed quinolone antibiotics for urethritis, gastrointestinal infections, sexually transmitted disease, respiratory infections, osteomyelitis and soft tissue infections. Levofloxacin is especially recommended for bronchitis, pneumonia, and sinusitis (Kathleen 2017).

Cefepime and ceftazidime are broad-spectrum cephalosporin drug which has high antibacterial activity against a broad range of Gram-negative bacteria, particularly Pseudomonas aeruginosa. (Gallagher and MacDougall 2016).

Carbapenems are broad-spectrum antibiotics and a cell wall synthesis inhibitors that have a similar mechanism of action to penicillin but are more resistant to beta-lactamases, making them an ideal therapy option. They work at very low concentrations and may be taken by mouth with just little side effects (allergies, for example). Imipenem inhibits a huge spectrum of Gram-positive and negative bacteria, Gram-negative rods and anaerobes, whereas
Meropenem kills more Gram-negative bacteria (Riedel et al. 2022).

Gentamicin and Amikacin belong to the aminoglycoside group and aminoglycoside is an inhibitor of protein synthesis.

Gentamicin is a less toxic antibiotic commonly used to treat gram-negative rod diseases; Amikacin is a new aminoglycoside that is used to treat gram-negative infections (Riedel et al. 2022).

Piperacillin /Tazobactum are beta-lactamase inhibitors which resemble beta-lactams structurally and bind to various beta-lactamases irreversibly, preventing them from inactivating the co-administered beta-lactam.

As a result, piperacillin/tazobactam inhibits *P. aeruginosa* growth. (Gallagher and MacDougall 2016).

As a consequence, these antibiotics might be helpful in hospitals, to treat *P. aeruginosa* infections, especially in individuals with weakened immune systems, such as those who are admitted to the burn unit or those with wounds or tracheal infections (Riedel et al. 2022).

By (Riedel et al. 2022) all of (Aminoglycoside + carbapenemes + an antipseudomonal penicillin), it was the medication of choice for Suspected or Proven Microbial Pathogens, (Table 2) explain the MICs results of the antibiotic and the activity (resistance or susceptible) for each antibiotics. The quality control organism was *Pseudomonas aeruginosa* ATCC® 27853TM. (for comprehensive quality control) and To classify organisms as susceptible or resistant, the (CLSI) or (the Clinical and Laboratory Standards Institute) standards were used to compare the results. (Figure 1).

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>CODE</th>
<th>ACTIVITY</th>
<th>MICs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefazolin</td>
<td>CZ</td>
<td>R (resistance)</td>
<td>&gt;=64</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>TGC</td>
<td>R(resistance)</td>
<td>&gt;=8</td>
</tr>
<tr>
<td>Piperacillin /Tazobactum</td>
<td>TZP</td>
<td>S (susceptible)</td>
<td>8</td>
</tr>
<tr>
<td>Amikacin</td>
<td>AMK</td>
<td>S (susceptible)</td>
<td>&lt;=2</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>CAZ</td>
<td>S (susceptible)</td>
<td>2</td>
</tr>
<tr>
<td>Imipenem</td>
<td>IPM</td>
<td>S (susceptible)</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GM</td>
<td>S (susceptible)</td>
<td>&lt;=1</td>
</tr>
<tr>
<td>Cefepime</td>
<td>FEP</td>
<td>S (susceptible)</td>
<td>&lt;=1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>LEV</td>
<td>S (susceptible)</td>
<td>0.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>S (susceptible)</td>
<td>&lt;=0.25</td>
</tr>
</tbody>
</table>

Figure 1. MICs percentage of antibiotics
4. CONCLUSIONS AND RECOMMENDATION

The ability of *P. aeruginosa* to easily colonize the host and its mechanism of action through virulence factors and genes made it a critical pathogen and needs care and monitoring before it worsens.

The data we obtained by this research show different clinical isolates from *P. aeruginosa* which have different resistance profiles were detected at Baghdad hospitals. It has been hypothesized that the high levels of antimicrobial resistance by cephalosporin like (cefazolin) and glycyclines like (Tigecycline) most likely are due to inappropriate doses that given to patients and that resistance is considerable because it limits the number of reliable antibiotics for treating patient with sever wound burned or lung infection.

There is Multiple factors are involved in the effect of antimicrobial therapies, especially since they may have been given to patients previously, and wrong instruction in using medical apparatus, in addition *P. aeruginosa* is a normal flora in human body and frequent entry into the body may help increase resistance to certain antibiotics through the formation of biofilms by the autoinducer in the controlled quorum sensing system, because the acquisition of resistance induce bacteria to be stronger and that helping bacteria to compete and assist in the colonization process.

We can reduce the resistance against Tigecycline and cefazolin by doing the susceptibility test to make the right choice (dose or concentration) and avoid the long therapy of the same antibiotics without any response from patient.

Wide research in our country and world should show the variety of resistance of *P. aeruginosa* bacteria profile and the relationship between biofilm development and the capacity of its virulence genes on the intense infection.

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Author contributions: Çankiri University conceived the study idea, supervised and drafted the manuscript, both authors read and approved and helped in editing the manuscript.

Conflicts of interest disclosure: The authors declare no conflicts of interest.

Data availability: dataset was generated and analyzed during this study and data sharing is applicable.

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