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Antibiotics susceptibility against *Pseudomonas aeruginosa* isolated from different infections

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***Abstract*---**In the present study, a total 70 samples were collected from different sites of infection which include 25(35.7%) samples from bronchial wash, 15 (21.4%) samples from urine, 15 (21.4%) samples from ear swabs, and 15 (21.4%) samples wound swabs), these samples have been collected and tested during period from March 2022 to June 2022. The results showed that, out of 70 samples, 62(88.5%) give positive culture, while 8(11.5%) samples were negative culture. Out of 62 positive culture on different types of growth media, and the bacterial was identified according to gram stain, biochemical tests and Vitek compact system, the results showed that, only 18(29.1%) isolates were related to *Pseudomonas aeruginosa*, while 44 (70.9%) samples were related to other types of microbial agents, these isolates which include 5/18(28%) isolates from bronchial wash, 4/18(22.1%) isolates from urine, 2/18(11.1%) isolates from ear swabs and 7/18(38.8%) isolates from wound swabs. To confirm the isolates of bacteria was used automated Compact Vitek-2 system use GN-ID cards which contained 64 biochemical tests. The results demonstrated that all (18) isolates were confirmed with ID massage confidence level ranging excellent (probability percentage from 94 to 99.7%, this technique was characterized by fast detection of bacteria. it was found that, *Pseudomonas aeruginosa* was found in all isolates that identified by biochemical test (100%). All the identified of *Pseudomonas aeruginosa* isolates were subjected to *in vitro* antibiotic susceptibility test by Kirby - Bauer disc diffusion method. Selective antibiotics are used to show their effect on *Pseudomonas aeruginosa* isolates such as

Imipenem, Amoxillin, Ceftriaxone, Ceftazidime, Naldixic acid, Trimethoprim, Amikacin, Ciprofloxacin, Gentamicin, Meropenem, Cefotaxime and Augmentin. The results compare according to Clinical Laboratory Standard Institute guidelines. Highest rate of resistance is seen to almost antibiotics used in present study against *Pseudomonas aeruginosa*, the high rate of antibiotic resistance were related to Ceftriaxone in 17(94%) followed by Amoxillin, Trimethoprim and Augmentin in 15(83.3%) of each one, Imipenem in 13(72%), 9(50%) of Amikacin and Cefotaxime, 6(33.4%) of Gentamicin and 4(22.3%) of Ciprofloxacin, while no *Pseudomonas aeruginosa* isolate (0%) were resistance to Meropenem. Aim to study: The aims of this study to antibiotics susceptibility against *Pseudomonas aeruginosa* isolated from Different Infection.

Keywords---*Pseudomonas aeruginosa*, antibiotics susceptibility, Compact VITEK-2 System, UTIs, wound infection.

Introduction

When antibiotics were originally introduced, they were thought to be a miracle medication. Unfortunately, most of the less expensive antibiotics lost their potency owing to bacterial resistance (1). To treat minor illnesses, expensive and complex antibiotics were introduced (2). *Pseudomonas aeruginosa* is an aerobic, non-fermenting Gram-negative bacillus that is usually seen in nosocomial infections (3). *Pseudomonas aeruginosa* acquires antibiotic resistance by a variety of processes, including multi-drug resistance efflux pumps, resistance genes, biofilm development, aminoglycoside modifying enzymes, and mutations in several chromosomal genes (4). *Pseudomonas aeruginosa* is one of the most prevalent bacterial infections seen in health-care settings (5). Despite breakthroughs in medical and surgical treatment, as well as the introduction of a wide range of antimicrobial medicines, *Pseudomonas aeruginosa* continues to produce potentially fatal infections (6). Infections caused by *Pseudomonas aeruginosa* are becoming more common in hospitals and the general community, and it has been identified as a leading source of nosocomial pathogens, particularly in immunocompromised patients (7). Concurrently, the widespread use of antimicrobial medications, as well as bacteria's evolving antimicrobial resistance mechanisms, have led in the creation of drug-resistant bacteria. Many antibiotics' efficacy in treating infections has become fairly restricted as a result of resistance development, and the threat from antimicrobial resistant organisms is increasing and accelerating (8). In critical care units (ICUs), *P. aeruginosa* was the second most prevalent cause of pneumonia, the fourth most common source of urinary tract infection, and the sixth most common blood stream isolate (9). Many possible infection reservoirs in the hospital environment have been discovered, including breathing equipment, cleaning solutions, disinfectants, sinks, vegetables, flowers, endoscopes, and hydrotherapy pools (10). Furthermore, broad-spectrum antibiotic exposure and patient-to-patient transmission have contributed to the fast rise in the isolation of resistant bacteria (11). Despite breakthroughs in health care and a vast range of antipseudomonal medications, life-threatening infections caused by *Pseudomonas aeruginosa* remain one of the

most serious public health issues (12). The emergence of infections caused by MDR and PDR strains increases morbidity and death while imposing a massive financial burden on health-care systems. Bacterial resistance patterns evolve throughout time and vary by location (13). As a result, both national and local surveillance is required to treat the illness empirically and effectively (14).

Materials and methods

A. Patients and collection of samples

The Cross Sectional study was carried out for a period of (3) month from March (2022) to June (2022). 70 sample were collected from different sites of infection which include (25 samples from bronchial wash, 15 samples from urine, 15 samples from ear swabs, and 15 samples wound swabs) from patients who visit Medical City Hospital in Baghdad, each samples were culturing on different types of culture media, and following standard procedure for microscopic examination and isolation of bacteria. Specimens were collected carefully to avoid any contamination. One aliquot of collected specimen was immediately inoculated in Blood, MacConkey and nutrient agar media at aerobic culture, then was incubated at (37^{o}C) for (24) hours aerobically cultured. Aerobic bacterial isolated were diagnosed by gram stain, colony morphology, biochemical test, and Compact VITEK-2 System for identification of *Pseudomonas aeruginosa*.

B. Ethical Approval:

A valid consent was achieved from each patients before their inclusion in the study.

C. Identification of bacterial isolates by gram stain, biochemical tests, Compact VITEK-2 System:

1. Identification of bacterial isolates by gram stain and biochemical tests:

The identification tests, including cultural, morphological and biochemical characteristics were done for each isolate according to (15; 16).

2. Identification of *Pseudomonas aeruginosa* isolates with Compact VITEK-2 System:

The Compact VITEK-2 System was used to screen and identify all *Pseudomonas aeruginosa* isolates (BioMerieux). This is a phenotypic identification method that relies on biochemical reactions to identify isolates. The Vitek-2 card has 64 wells for various fluorescence biochemical tests. Phosphatase, urea, nitrate, and actidione tests were 20 of the 64 carbohydrate absorption tests. The Vitek-2 machine autonomously managed the card, including filling, sealing, and transporting the cards into the attached incubator (35°C). Each output report is decoded using a specific algorithmic technique. The obtained findings were recognized using the ID-GN databank (identification of Gram-negative bacteria). The IDs generated by these systems are automatically proposed by the supporting software. Only if the first findings indicated "poor discrimination" or "no ID" were the tests repeated, and the repeat result was utilized for data analysis. All strains were put into culture medium and incubated at 37°C overnight. The phenotypic VITEK-2 Systems approach was used to identify a single isolated colony, following the manufacturer's instructions (BioMerieux).

D. Antibiotics Susceptibility Testing (Disk Diffusion Test):

It was carried out with the help of a pure culture of a previously described bacterial organism. The inoculum for this test was made by combining growth from 5 isolated colonies grown on blood agar plates with 5 ml of nutrient broth; this culture was then incubated for 2 hours to produce a bacterial suspension with moderate turbidity when compared to the turbidity of a ready-made 0.5 McFarland tube standard. To extract an inoculum from the standardized culture, a sterile swab was employed; this inoculum was then swabbed on Muller–Hinton agar plate.

1. The antibiotic discs were put on the surface of the medium with flamed forceps at regularly spaced intervals, then incubated at 37oC for 18 hours before reviewing the data to detect cells displaying hetero-resistance.
2. A transparence ruler was used to assess antibiotic inhibition zones. The zone size was compared to typical zones to evaluate the organism's sensitivity to each antibiotic (17).

Results

In the present study, a total 70 samples were collected from different sites of infection which include 25(35.7%) samples from bronchial wash, 15 (21.4%) samples from urine, 15 (21.4%) samples from ear swabs, and 15 (21.4%) samples wound swabs), these samples have been collected and tested during period from March 2022 to June 2022. The results showed that, out of 70 samples, 62(88.5%) give positive culture, while 8(11.5%) samples were negative culture as shown in Figure (1). Out of 62 positive culture on different types of growth media, and the bacterial was identified according to gram stain, biochemical tests and Vitek compact system, the results showed that, only 18(29.1%) isolates were related to *Pseudomonas aeruginosa*, while 44 (70.9%) samples were related to other types of microbial agents. the results were shown in Table (1), these isolates which include 5/18(28%) isolates from bronchial wash, 4/18(22.1%) isolates from urine, 2/18(11.1%) isolates from ear swabs and 7/18(38.8%) isolates from wound swabs as shown in Table (2). To confirm the isolates of bacteria was used automated Compact Vitek-2 system use GN-ID cards which contained 64 biochemical tests. The results demonstrated that all (18) isolates were confirmed with ID massage confidence level ranging excellent (probability percentage from 94 to 99.7%, this technique was characterized by fast detection of bacteria. it was found that, *Pseudomonas aeruginosa* was found in all isolates that identified by biochemical test (100%).

Table (1): identification of *Pseudomonas aeruginosa* from Positive culture in this study

No. of samples	Positive culture	Negative culture	*Pseudomonas aeruginosa*	other types of microbial agents
70	62(88.5%)	8(11.5%)	18(29.1%)	44(70.9%)
Total	**70(100%)**		**62(100%)**	

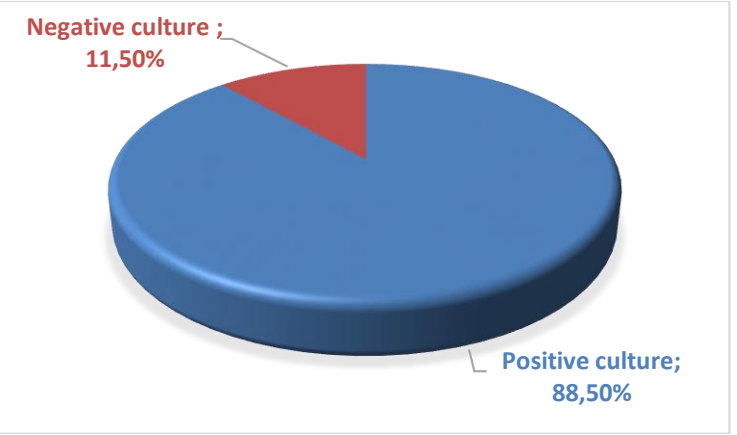

Figure (1): Positive and negative culture of all samples from different sites of infection

Table (2): Distribution of *Pseudomonas aeruginosa* from different sites of infection

No.	**Site of infection**	**No. of samples**	**%**	***Pseudomonas aeruginosa* No. 18 (%)**
1.	Bronchial wash	**25**	35.8	5/18(28%)
2.	Urine	**15**	21.4	4/18(22.1%)
3.	Ear swabs	**15**	21.4	2/18(11.1%)
4.	Wound swabs	**15**	21.4	7/18(38.8%)
Total		**70**	**100**	**18**

All the identified of *Pseudomonas aeruginosa* isolates were subjected to *in vitro* antibiotic susceptibility test by Kirby - Bauer disc diffusion method. Selective antibiotics are used to show their effect on *Pseudomonas aeruginosa* isolates such as Imipenem, Amoxillin, Ceftriaxone, Ceftazidime, Naldixic acid, Trimethoprim, Amikacin, Ciprofloxacin, Gentamicin, Meropenem, Cefotaxime and Augmentin. The results were are shown in Figure (2). The results compare according to Clinical Laboratory Standard Institute guidelines (CLSI, 2019). Highest rate of resistance is seen to almost antibiotics used in present study against *Pseudomonas aeruginosa*, the high rate of antibiotic resistance were related to Ceftriaxone in 17(94%) followed by Amoxillin, Trimethoprim and Augmentin in 15(83.3%) of each one, Imipenem in 13(72%), 9(50%) of Amikacin and Cefotaxime, 6(33.4%) of Gentamicin and 4(22.3%) of Ciprofloxacin, while no *Pseudomonas aeruginosa* isolate (0%) were resistance to Meropenem.

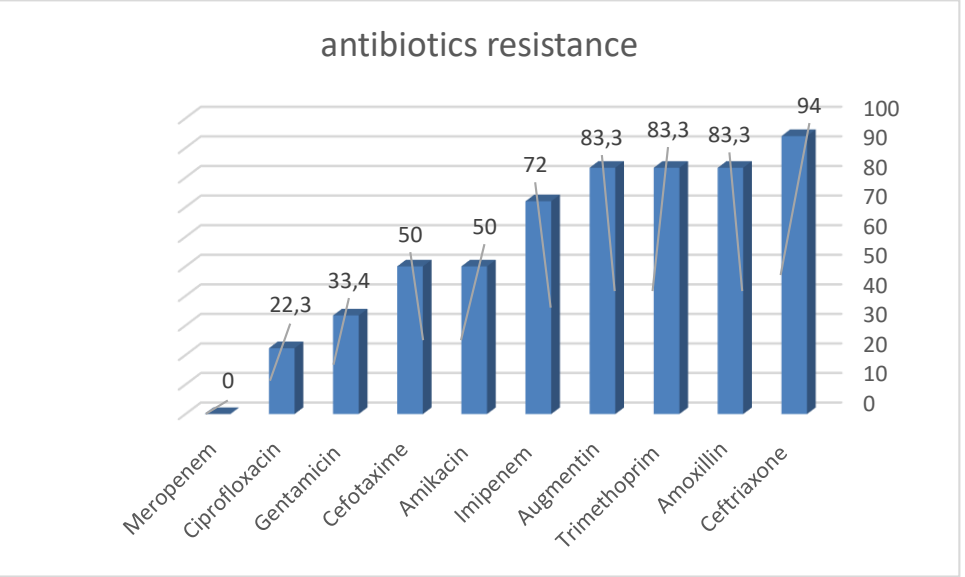

Figure (2): Antibiotics resistance of *Pseudomonas aeruginosa* isolates

Discussion

P. aeruginosa is an important opportunistic pathogen that causes many fatal infections in people with major medical problems, such as those who are immunocompromised (18). *P. aeruginosa* is regarded as extremely harmful due to its ability to colonize epithelial surfaces, undermine host defenses, generate systemic toxicity, and be related with increased morbidity and death rates (19). Antibiotic therapy is critical for treating severe *Pseudomonas* infections (20). Antibiotic resistance in Pseudomonas species has increased significantly in recent years, necessitating frequent evaluation in order to have a clear judgment on the clinical result of various treatment choices (21). *Pseudomonas aeruginosa* is the second most frequent gram negative bacteria isolated from catheterized patients suffering from UTI, wound infection, and respiratory illnesses (22). In the current investigation, the prevalence of *P. aeruginosa* was reported to be 29.1%. In comparison to Motbainor *et al.*, (23) who discovered that this Bacteria was present in (27%). Furthermore, the length of hospital stay and catheterization are important factors (24). Tong *et al.*, (25) discovered a total of 73 catheterized patients recruited. These patients' urine specimens were tested using conventional laboratory procedures, and 36 (49.32%) cultures were found positive for *P. aeruginosa*. In another investigation, Samrot *et al.*, (26) discovered that 19 (26.03%) of positive cultures for P. *aeruginosa* were identified from urine specimens. *P. aeruginosa* antimicrobial susceptibility patterns to routinely used medications Ceftriaxone resistance was found in 17 (94%) of *P. aeruginosa* strains, followed by Amoxillin, Trimethoprim, and Augmentin resistance rates (83.3%). Mohamed and Abdelhamid, (27) discovered that *P. aeruginosa* antibiotic resistance profile revealed diverse antibiotic resistance patterns to various drugs. Bacterial resistance to ciprofloxacin (63%), gentamycin (62%), and meropenem was found to be high (60 %). *Pseudomonas aeruginosa* is a regularly isolated bacteria that is growing increasingly resistant to commonly used medications. Carbapenems and aminoglycosides were the two medication families that shown

the most activity against *Pseudomonas* (28). Gentamicin, in particular, is a well-known first-line antibiotic for the treatment of gram-negative bacterial infections. However, Walker *et al.*, (29) discovered an increase in the incidence of *Pseudomonas* resistance to these medicines. Improper antimicrobial usage led in the formation of MDR strains, which are difficult to treat because to their increased resistance to multiple antibiotics (30, 31). The acquired results might be linked to a variety of variables that contributed to the expansion of MDR isolates, the most prominent of which is the growing catastrophe of antibiotic overuse without valid prescriptions (32). This increased MDR incidence serves as a warning that strict antibiotic prescribing practices are required (33).

Conclusion

Pseudomonas aeruginosa is a widely isolated bacteria which is growing increasingly resistant to commonly used medications. The classes of medications with the strongest resistance to Pseudomonas aeruginosa were Ceftriaxone, Amoxillin, Trimethoprim, and Augmentin, whereas Meropenem was very susceptible.

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