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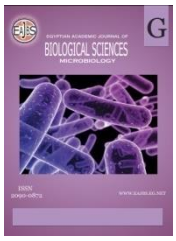

Frequency of Metallo-B-Lactamase Among *Pseudomonas aeruginosa* Isolated from patients In Intensive Care Units and Operating Rooms

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ABSTRACT

Background: *P.aeruginosa* infection is one of the major health problems in the world. It's an opportunistic human pathogen that causes serious problems. Usually resistant to several antibiotics, show a particular ability to spread in hospitals. In recent times, Metallo-β-lactamase resistance in this bacterium has imposed some difficulties in treating bacterial infections. This study was a qualitative study, aimed to detect Metallo-β-Lactamase in Carbapenems-resistant P. aeruginosa isolated from inpatients admitted to Intensive Care Unit and Operating Room in different hospitals in Jeddah city, KSA during the period from March 2019 to September 2022.**Methods:** In this study, a total of (234) were cultured and identified using API 12A/12E. All isolates were subjected to antimicrobial susceptibility testing using Kirby Bauer method, for selected imipenem and meropenem. **Results:** Out of 234 specimens 154 *Pseudomonas aeruginosa* were isolated and identified, with different ratios (males, 62.3: females, 37.7), which occurred highest in the adult age group. The highest frequency of isolate 152 (98.7%) was in the intensive care unit while the lowest frequency of isolate 2 (1.3%) was in the operating room. (9%), (5.8%), (10.38%) and (1.2%) were found resistant to imipenem, meropenem, ciprofloxacin and amikacin respectively.**Conclusion:** Carbapenems have great bactericidal activity against *Ps. aeruginosa*, while, this notorious pathogen acquisition resistance against these drugs and limited treatment options. Our isolated strains showed a low rate of resistance (9%) and (5.8%) against imipenem and meropenem respectively. Aminoglycoside is crucial for the treatment of various *Ps. aeruginosa* infections. However, our study showed that (1.2%) of *Ps. aeruginosa* strains were resistances to amikacin and Ciprofloxacin has been extensively used to treat wide a variety of *Ps. aeruginosa* infections. While *Ps. aeruginosa* rapidly acquired resistance to ciprofloxacin that creating a therapeutic challenge. In this study, 10.38% of *Ps. aeruginosa* was found resistant to Ciprofloxacin. We recommended further studies and a regular monitoring system for the early detection of MBL-producing organisms.

INTRODUCTION

Antibiotic resistance is a worldwide problem of major importance. In fact, numerous studies highlight the link between multi-drug resistance and increased morbidity and mortality, increased length of hospital stay and higher hospital costs. (Meletis & Bagkeri, 2013; Roca, *et al.* 2015). Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are among the most important causes of serious hospital-acquired and resistance in these bacteria have become a growing problem (Memish, *et al.*, 2014) the increasing prevalence of chronic and hospital-acquired infections produced by (MDR) or (XDR) *Pseudomonas aeruginosa* strains is associated with significant morbidity and mortality. Indeed, recent concerning reports have provided evidence of the existence of MDR/XDR global clones, denominated high-risk clones, disseminated in hospitals worldwide (Oliver, *et al.* 2015) *P. aeruginosa* is able to readily develop resistance to a number of commonly used antibiotics, including first-line antipseudomonal agents. As such, *P. aeruginosa* is one of the most commonly isolated carbapenem-resistant (CR) Gram-negative bacteria encountered in the hospital with difficult-to-treat resistance (DTR; exhibiting non-susceptibility topiperacillin–tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem-cilastatin, ciprofloxacin, and levofloxacin) (Canton, *et al*. 2022).

MATERIALS AND METHODS

Out of 234 specimens 154 *Pseudomonas aeruginosa* were isolated from patients with different clinical manifestations who attended different hospitals in Jeddah, KSA. Specimens were wound, ear swabs and eye swabs urine samples, tracheal aspiration, pus, blood, sputum, and Necrotic tissue. Urine samples were collected after instructing the patients to collect a midstream urine sample (MDS) in a sterile container in the correct way and from the catheter bags. Wound, eye and ear swabs were done by experienced nurses and doctors using a sterile swab. Necrotic tissue, tracheal aspiration, pus, and blood samples were collected by doctors. All clinical specimens were collected in sterile containers under aseptic conditions according to the recommendations of the Clinical and Laboratory Standards Institute.

Phenotypic Characterization:

A standard scheme for identifying all *Pseudomonas aeruginosa* was used (Cheesbrough, 2007). These include colony morphology on blood agar, CLED agar, MacConkey agar and Nutrient agar, Gram-stain and different biochemical tests were also used (citrate and oxidase test).

Determination of Antibiotic Susceptibility Profile:

All isolated organisms were tested for their in-vitro antimicrobial susceptibility against various antibiotics using the Kirby-Baur disk diffusion method according to the CLSI (Clsi, 2010). 2-3 fresh colonies were suspended in 1ml nutrient broth and adjusted to 0.5 Mac Farland standard tube. All antibiotic discs used in this study were listed in Table (1). Control strain was also used in this regard (ATCC 27853). Antibiotic sensitivity discs were placed on each plate of Mueller-Hinton agar and then incubated at 37°C for 24 hours. The plates were examined for zones of inhibition around each antibiotic disc. These were measured and compared with an interpretive chart to determine the sensitive and resistant strains.

Table 1. Antibiotic Discs uses in the Study.

Antibiotic discs	Drug Class	Doses	Doses
Amikacin	Aminoglycosides	(30) mcg	(AK 30)
Ciprofloxacin	Quinolones	(5) mcg	(CIP 5)
Imipenem	Carbopenems	(10) mcg	(IMP 10)
Meropenem		(10) mcg	(MEM 10)

Automated Methods:

Biomerieux VITEK 2 System:

The VITEK 2 is an automated microbiology system utilizing growth-based technology. The procedure could be summarized as follows:

Choose clear Isolate then prepare organism suspension and ensure correct McFarland Standard according to company guidelines with (Densichek® Plus) With VITEK 2/XL scan card by barcodes scanner and Isolate barcodes to establish traceability then load cards on the instrument for fully automated processing. For VITEK 2 Compact: ID suspension was used to make antibiotic susceptibility testing (AST) suspension then, cards were inoculated inside the instrument and manually transferred from the filling door to the loading door for processing, scan cassette worksheet at the workstation. Finally, Results in as little as 5 to 8 hours for identification and 10 to 18 h for AST.

Ethical Clearance

The proposal for this study was submitted to the Federal Ministry of Health as well as the College of Medical Laboratory Science at Kordofan University for ethical approval. A form of consent was taken by patients participating in the study.

RESULTS AND DISCUSSION

Demographic Data:

A total of (154) clinical specimens were collected from patients with different clinical lesions including (wound, ear and eye swabs, urine samples, tracheal aspiration, pus, blood, sputum, and Necrotic tissue). Specimens were collected from different Hospitals, these include Saudi German Hospital Jeddah (50), King Fahad Armed Forces Hospital (70), Suliman Alhabib Hospital (16), Jeddah National Hospital (7), and King Khalid Hospital (11). Among the study population, 96 (62.3%) were males while 58 (37.7%) were females, among these *P. aeruginosa* was identified in all patients as shown in (Fig. 1).

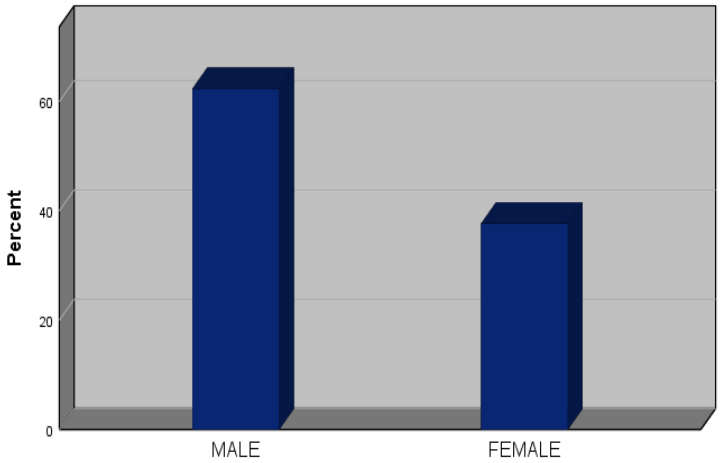

Fig.1: Distribution of study population by gender

Enrolled Patients Versus Age Groups:

Patients enrolled in the study were divided into three age groups: young less than (1 yrs.), children (1- 16 yrs.), and adults more than (16 yrs.). The highest frequency of isolates 146 (94.8%) was in the adults' age group of more than (16 yrs.), followed by the child age group of (1-16 yrs.) and the young age group of less than (1 yrs.) by 4 (2.6%) as shown in (Fig. 2). The different rate age group statistically significant at p-value=0.000.

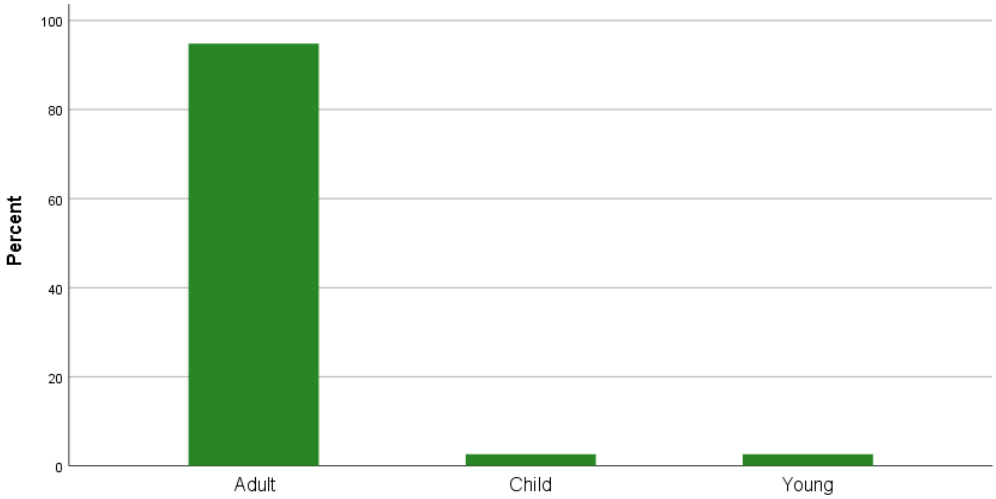

Fig. 2: Frequency of the isolates according to age groups **(**p-value=0.000)

Enrolled Patients Versus Clinical Units in Hospitals:

In this study based on clinical units in hospitals were divided into two groups ICU and OR. The highest frequency of isolate 152 (98.7%) was in the intensive care unit while the lowest frequency of isolate 2 (1.3%) was in the operating room as shown in (Fig. 3).

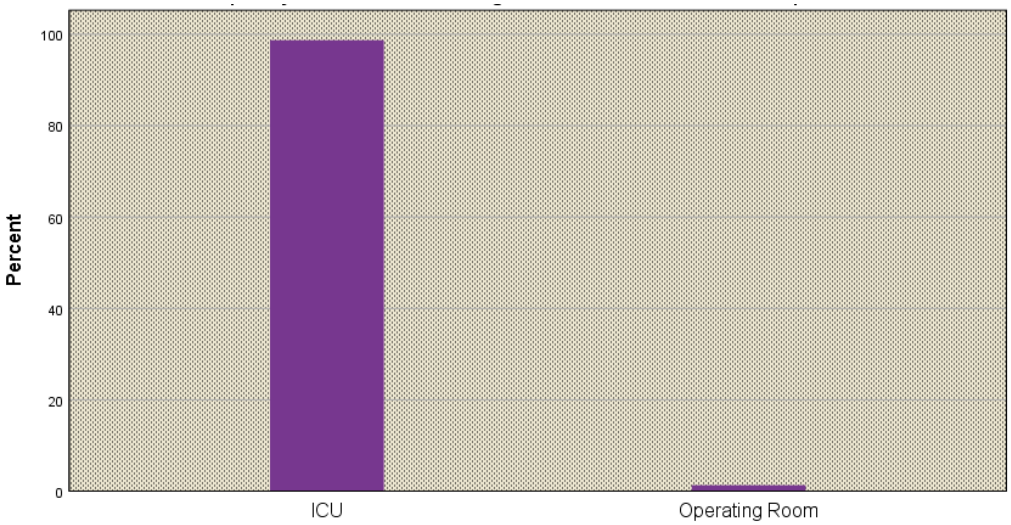

Fig. 3: Frequency of the isolated bacteria from clinical units in hospitals.

Bacteriological Findings:

Identification Scheme:

The bacterial isolates obtained in this study were identified according to their cultural characteristic, colonial morphology, Gram reaction and biochemical properties, the total number of bacterial isolates were (154), The identification scheme confirmed that all isolates were *P. aeruginosa*, the colonies were large, opaque, some are mucoid, flat colonies with irregular margins and distinctively fruity odor colonies on nutrient agar showing greenish coloration. All strains were motile and gave positive results for Oxidase and Citrate tests. The majority (32.5%), of isolated *P. aeruginosa* strains, were from urine, (18.2%) wounds, (13.6%) ear swabs, (13%) sputum, (8.4 %) blood, (5.8%) pus, tracheal aspiration (5.2%) and minority (1.9%) from necrotic tissue. Only (1.3%) of isolates were related to eye swabs (Figs. 4, 5, 6 and 7).

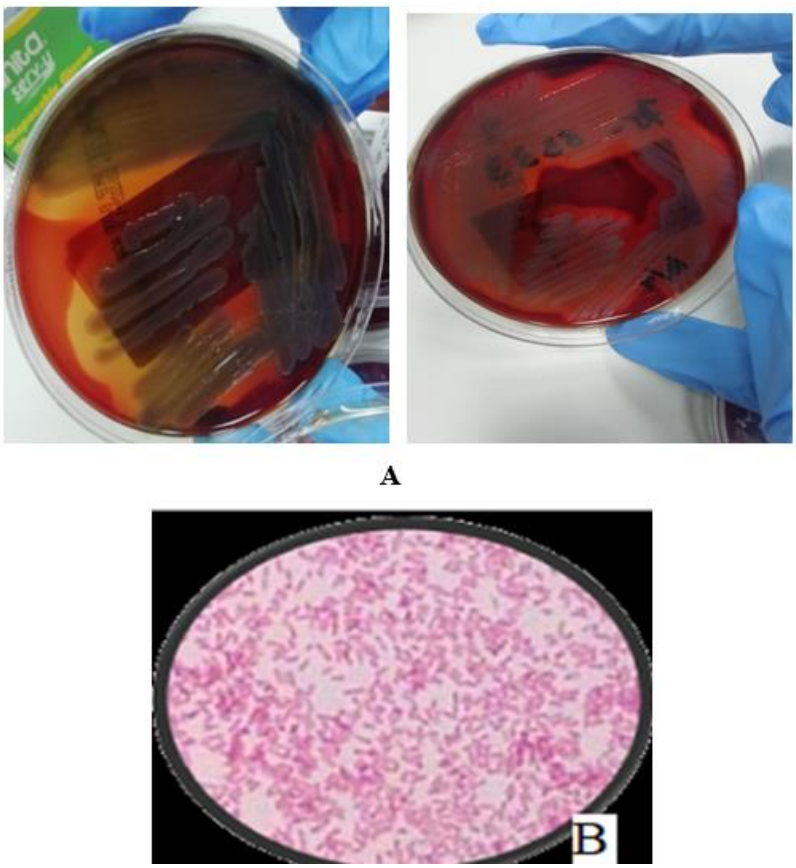

Fig. 4: Identification of the *P. aeruginosa* (A); Overnight growth of *P.aeruginosa* on blood agar medium, (B); *P.aeruginosa* under a microscope with X100 objectives.

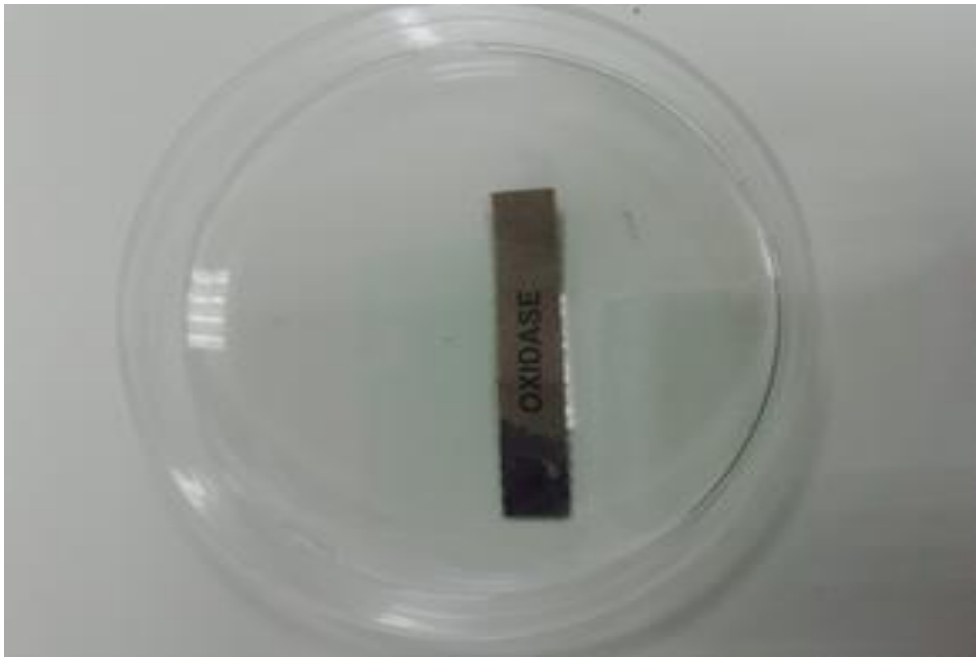

Fig. 5: Cytochrome oxidase enzyme produced by *P. aeruginosa*

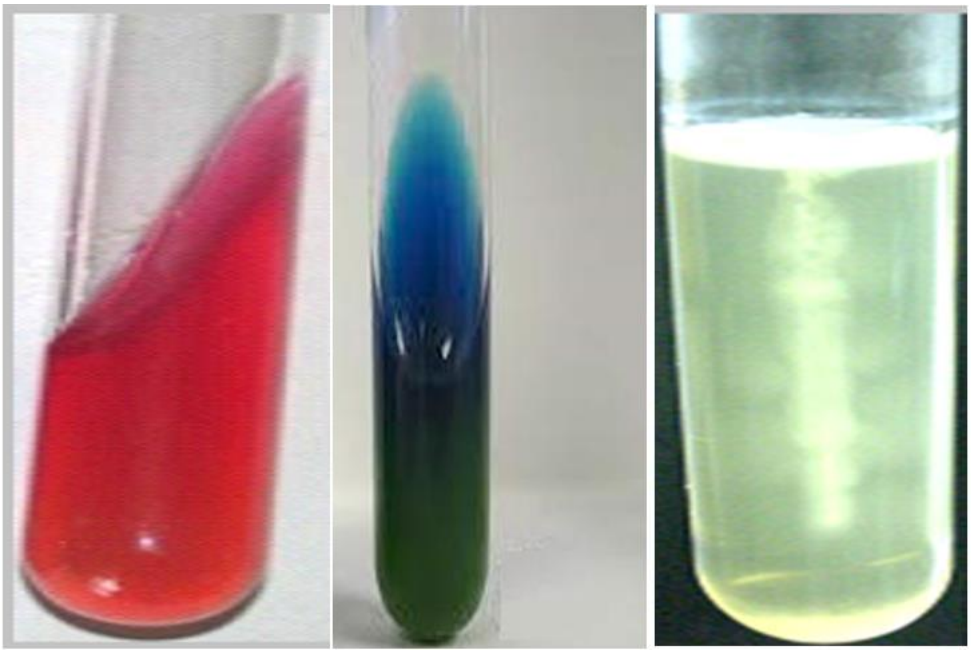

Fig. 6: Biochemical test of *P.aeruginosa* (KIA none lactose and glucose fermented without gas and H_2s, citrate positive and motile bacteria).

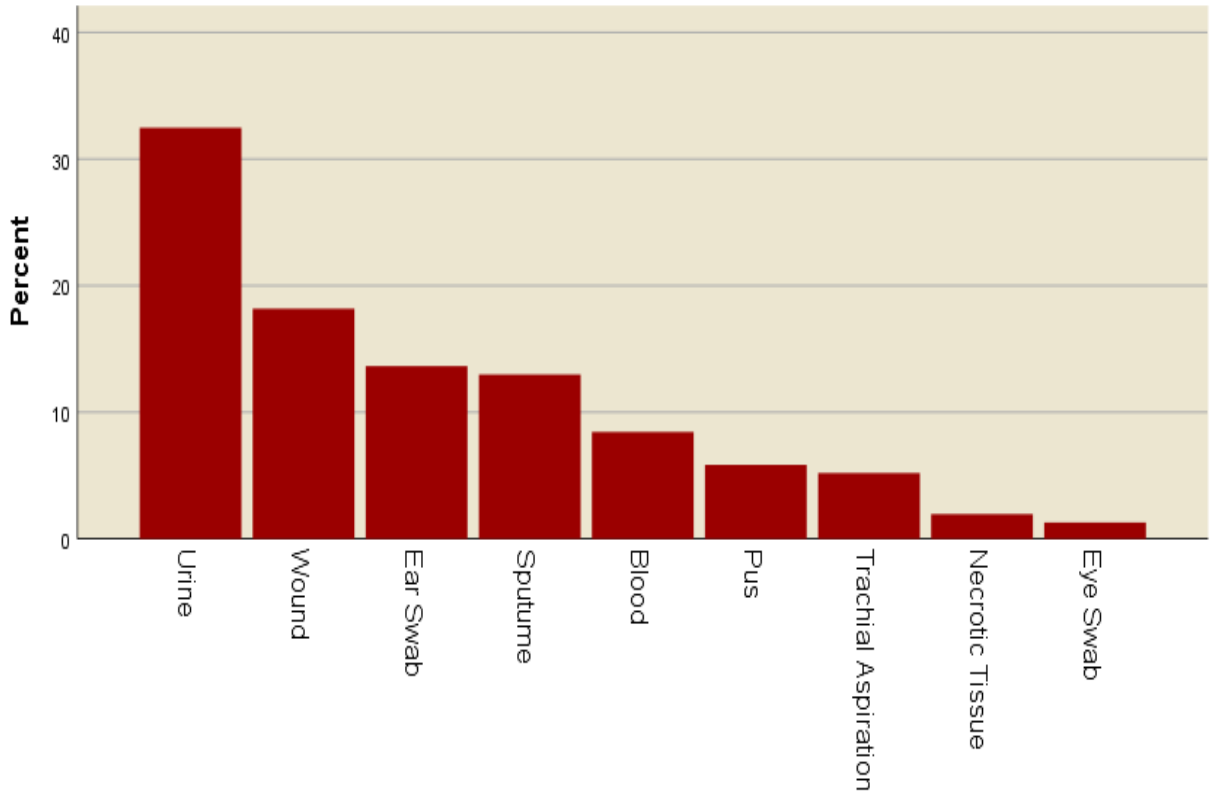

Fig.7: Frequency of the isolated bacteria from clinical samples.

Results of Modified Kirby-Bauer Technique:

The results of the modified Kirby-Bauer method showed that 20 (13%) of isolated *P. aeruginosa* were resistant to imipenem, meropenem, amikacin and ciprofloxacin while 134 (87%) were sensitive as shown in (Figs. 8 and 9). The different rates of sensitivity tests were statistically significant at p-value=0.000.

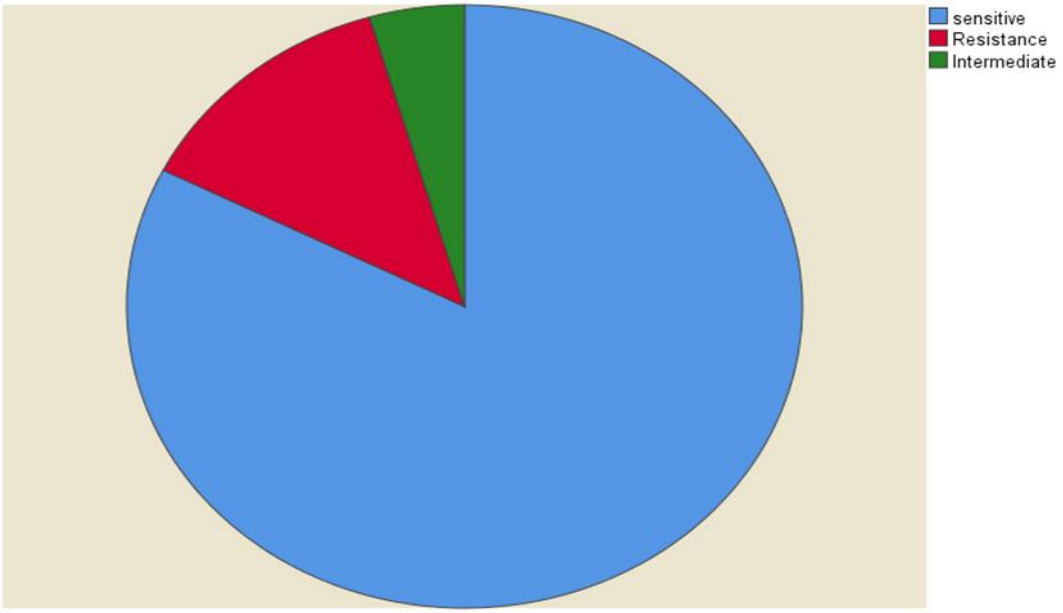

Fig.8: Antimicrobials susceptibility patterns of isolated bacteria.

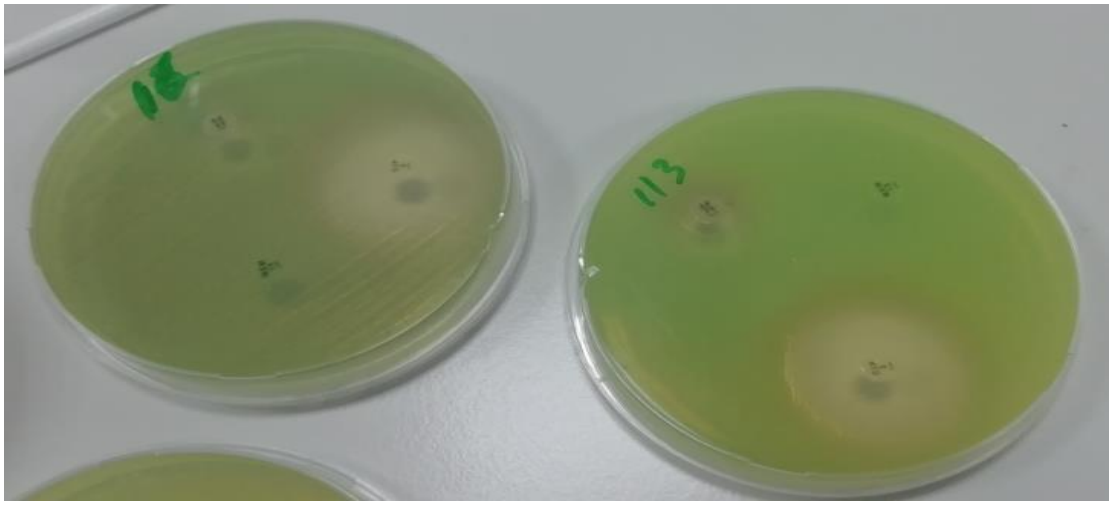

Fig. 9: Susceptibility test of *P. aeruginosa* by modified Kirby-Bauer method.

Table (5) showed the different antibiotic susceptibility patterns of all tested *P. aeruginosa* isolates using the disk diffusion method. 4 different antibiotic susceptibility patterns were detected among tested *P. aeruginosa* strains. All samples were susceptible to amikacin except 2 (1.2%) strains No. 41 and 143. All strains were susceptible to imipenem except 14 (9%) strains No. 7, 31, 37, 41, 49, 52, 54, 55, 110, 118, 119,121,139 and 154. All strains were susceptible to meropenem except 9 (5.8%) strains No.31, 41,43,52,54,110,118,119 and 121. All strains were susceptible to ciprofloxacin except 16 (10.38%) strains No. 7, 21, 28, 31, 39, 46, 48, 52, 54, 110, 121, 127, 139, 143, 150 and 152.

Table 5: Antibiotic sensitivity patterns of the isolated 20 strains of *PA.*

Strain(s) No.	Antibiotic sensitivity patterns				No. of strains
	IMP	MER	AMK	CIP	
7,139	R	S	S	R	2
31,52,54,110,121	R	R	S	R	5
41	R	R	R	S	1
118,119	R	R	S	S	2
143	S	S	R	R	1
21	S	S	S	R	1
28	S	S	S	R	1
37	R	S	S	S	1
39	S	S	S	R	1
43	S	R	S	S	1
46	S	S	S	R	1
48	S	S	S	R	1
49	R	S	S	S	1
55	R	S	S	S	1
127	S	S	S	R	1
150	S	S	S	R	1
152	S	S	S	R	1
154	R	S	S	S	1

In the current study, the evidential microbiological diagnosis was made by isolation of bacteria from 154 different clinical samples that resulted from *P.aeruginosa* isolates, the numbers of isolates similar to that isolated in studies reported in several countries, including Shifa Hospital, India by (Ali, *et al*.2020) they collect150 strains, in Sudan by (Elbadawi, *et al*.2019) who recorded that *Pseudomonas aeruginosa* 153 isolates, (YAGOUP, *et al*.2018) who collect 150 clinical samples and by (Badri, A. M., & Mohamed, S. G. 2017) also they collect 150 samples. Our study is in disagreement with a study conducted by (Badger, *et al*.2018) in KSA where a total of 580 samples were used for the investigation, from Deeba, *et al*. (2011) in India 283 samples and also from Amoon *et al* (2018) Sudan have used 40 clinical isolates. This variation in the prevalence rate of *P.aeruginosa* presented by different studies might be attributed to the type and size of clinical specimens, studied populations, hospital situations and geographical locations.

The study also confirmed that high prevalence of *P.aeruginosa* among males compared to females (62.3%: 37.7%) These findings semi agreement with a previous study conducted in north-central Nigeria were males (50.3%) and females (49.7%) by (Ndukwe, *et al*.2021) and also with a study carried out in KSA were (52%) of the specimen were from males while the remaining (48%) were from females (Badger, *et al*.2018).

In this study, the findings suggested a high-frequency prevalence of *P.aeruginosa* among the adult's age group (94.8%) these findings agree with a previous study conducted in Nigeria by (Ndukwe, *et al*.2021) who recorded that infection was found to be high among these variables; the older age group.

P.aeruginosa commonly infected all ages, very old patients had higher rates of infection overall than did other age groups, but the risk of infections in different sites changed significantly with age, changes in hormonal status, a decline in the immune system, malnutrition, functional disability, and coexisting illnesses (Bennett, J. V. 1974; Luiz *et al*., 2012).

The results obtained from this study suggested a frequency of (98.7%) of *P.aeruginosa* infections occur in the ICU which is almost neighboring to the results obtained by Pachori, *et al* (2019) in India which demonstrated *P. aeruginosa* is a major pathogen in ICU and in Saudi Arabia (95%) by Al-Hussain *et al*. (2021). However, in this study, the association of *P.aeruginosa* with ICU infection is higher than in most of the studies done around the globe; in Saudi Arabia (41%) by Said, *et al*. (2021), in Mexico (30.41%) by Uc-Cachón *et al*. (2019), in Italy (29.9%) by Agodi, *et al*. (2007) and in United States Lob *et al*. (2021) also suggested the lowest frequency (27.9%) of *P.aeruginosa* that capable for causing infection in ICU.

ICUs are the major source of creating, disseminating and amplifying these drugs resistant organisms where the selection pressure is highest for the emergence of resistance to drug-resistant pathogens.

The ability of most *P. aeruginosa* strains to form biofilms and adhere to urinary catheters and urothelium makes urinary catheterized patients at high risk for developing UTIs (Elbargisy, *et al*. 2021). In this project, the majority of isolated *P. aeruginosa* strains were from urine (32.5%), which was similar to the results obtained by Amoon *et al* (2018) from Sudan (32.5%) and by Elbargisy *et al* (2021) from Egypt (50 isolates) and almost nearby to the results obtained by Ahani Azari, A., and Fozouni, L. (2020) from Iran (30%). But the low frequency of *Ps. aeruginosa* was reported by YAGOUP *et al,* (2018) from Sudan (8%) among Sudanese populations. This frequency was different than that documented in several countries, including Sudan (14%) by Badri, *et al*. (2017), India (12.4%) by Deeba, *et al*. (2011) and Iraq (10%) by Hasan, *et al*. (2020).

The results obtained from this study suggested a frequency of (18.2%) from wound isolates which are nearby to (22.2%) of the results obtained by Altamimi, *et al*. (2022) from Saudi Arabia. Only (1.3%) of isolates were related to swabs which is resemble to study conducted by Altamimi, *et al*. (2022) in KSA only (1%) was from swabs. Carbapenems have great bactericidal activity against *Ps. aeruginosa*, while, this notorious pathogen acquisition resistance against these drugs and limited treatment options. Our isolated strains showed a low rate of resistance (9%) and (5.8%) against imipenem and meropenem respectively this degree of resistance resembled to rate (8%) of imipenem resistance reported in Sudan by YAGOUP *et al* (2018). This degree of resistance was lower than that documented in several studies (13.42%) in India by Deeba, *et al*. (2011), (64.6%) Sudan by Badri, *et al*. (2017) and in KSA (36.7 %) by Ahmad *et al*. (2020). This might be attributable to the recent introduction of carbapenems in treatment policy in our hospitals and still low consumable drugs due to their high cost.

Aminoglycoside is crucial for the treatment of various Ps. aeruginosa infections. However, our study showed that (1.2%) of *Ps. aeruginosa* strains were resistances to amikacin which is lower than that documented in Sudan (13.5%) by YAGOUP *et al,* (2018) and in KSA (43.3%) by Ahmad *et al*. (2020).

Ciprofloxacin has been extensively used to treat wide a variety of *Ps. aeruginosa* infections. While *Ps. aeruginosa* rapidly acquired resistance to ciprofloxacin that creates a therapeutic challenge. In this study, 10.38% of *Ps. aeruginosa* was found resistant to Ciprofloxacin, which was nearby to the resistance rate (13.3%) reported in Saudia Arabia by Ahmad *et al.* (2020).

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