Antibiotic Resistance Patterns of *Pseudomonas aeruginosa* in Clinical Specimens from Khartoum Hospitals, Sudan

Abstract

Background: One of the most common causes of nosocomial infection is *Pseudomonas aeruginosa*. Antimicrobial resistance has expanded globally as a result of profound changes in microbial genetic ecology caused by the indiscriminate use of antimicrobials.
Objective: The aim of study was to determine the antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from clinical specimens.
Material and Methods: The present study used purposive sampling as the sampling technique. *Pseudomonas aeruginosa* isolated from 77 clinical specimens, Susceptibility Test was determined using Kirby-Bauer Disk Diffusion.

Results: The results revealed that and the isolated organism test is *Pseudomonas aeruginosa* was isolated from all the clinical Specimen by 100%, The results revealed that the frequencies of Cefpodoxime antibiotics for the Urine 32.4% (11/31) was sensitive, while 58.8% (20/31) was resistant., for the wound 20.6% (7/34) was sensitive, while 79.4% (27/34) was resistant., for the Ear 5.9% (2/12) was sensitive, while 29.4% (10/12) was resistant. Resistant average for cefpodoxime was 74.0%, and there is a high statistically significant relation between Cefpodoxime antibiotics and samples (Urine and wound) and a normal relation with Ear.

Conclusion: Drug resistance in *P. aeruginosa* is a multifactorial increasing phenomenon. Estimated frequencies were between 12-36%. Mechanisms of resistance either through membrane permeability and efflux system or through its virulence factors or acquired genetically by plasmid. Combination of these mechanisms leads to a superbug, which is very difficult to be treated.

Keywords: Prevalence, Multidrug-Resistant (MDR), *Pseudomonas aeruginosa*, nosocomial infection.

Introduction:

"One of the most common causes of nosocomial infection is *Pseudomonas aeruginosa (P. aeruginosa)"* [(1)]. "In clinical samples, multidrug-resistant *P. aeruginosa* (MDR PA) is becoming more common" [(2)]. "The pathogenic species *Pseudomonas aeruginosa* is the most common in the *Pseudomonaceae* family. *P.aeruginosa's* capacity to grow with minimum food requirements and its tolerance to a wide range of circumstances has allowed it to thrive in both hospitals and the community. *P. aeruginosa* is widely found in soil, water, and plants outside of the hospital" [(3)]. "*P. aeruginosa* can colonize moist areas on patients' bodies, as

well as most inanimate objects in the environment, such as water in sinks, drains, toilets, showers, mops, respiratory ventilators, and cleaning solutions. In Europe, 11.5 percent of people had *P. aeruginosa* infections, compared to 17 percent in underdeveloped countries. *P. aeruginosa* is one of the resistant bacteria, expressing resistance to antibiotics that can be acquired (plasmids, transposons) or natural" [(4)]. "This resistance favors *P. aeruginosa's* involvement in nosocomial infections, food poisoning, and biofilm formation, the latter conferring high colonization potential, the ability to spoil foods, and resistance to antiseptics, disinfectants, and antibiotics to *P. aeruginosa*. In addition, *Pseudomonas aeruginosa (Ps. aeruginosa)* is the leading cause of health-care acquired infections. It is most commonly associated with ventilator-associated pneumonia, urinary tract infections, wound infections and eye infections" [(5)]. "In addition, Ps. aeruginosa can be disseminated from the primary site of infection via blood causing serious metastatic infections such as septicemia, meningitis and brain abscess" [(6).] "On the other hand, Antibiotic resistance has been a serious communal health problem since the era of the discovery of antimicrobial drugs. Recently, the emergence of strains with resistance to multiple classes of antibiotics has complicated the decision for the selection of proper drugs" [(7)]. "Organisms become resistant to all available antimicrobial agents and are susceptible only to older, likely more toxic antimicrobials, leaving less effective scanty alternatives" [(8)]. "The Centers for Disease Control and Prevention (CDC) declared that worldwide increasing infection rates with resistant pathogens strikingly endanger our healthcare systems, creating both negative universal economic effects and a therapeutic challenge for clinicians, hence delaying proper antibiotic therapy and increasing mortality rates" [(9)]. "To combat this horrifying ascent in antimicrobial resistance, the World Health Organization (WHO) urges healthcare providers to adopt antimicrobial stewardship to decrease the heavy cargo of antibiotic resistance. However, before the implementation of any stewardship program, information on Prevalent MDRO and their antimicrobial resistance profile are required. Antimicrobial resistance is rapidly becoming a global focus of attention, especially with the rising number of microorganisms resistant to available antimicrobials. It encompasses both the gram-positive and gram-negative bacteria, with global prevalence rates of 60% or more" [(10)].

"Multidrug-resistant organisms (MDROs) are described as acquired non-sensitivity to one or more agents in at least three groups of antimicrobials. This kind of resistance essentially predominates in hospitals. The lack of quick, proper identification of pathogens, especially in patients with critical infection, led to broad-spectrum antibiotics overuse" [(11)]. "Data about the endemic antimicrobial resistance are generally difficult to find, particularly in countries where antibiotics are easily obtainable over the counter" [(12)]. "Although numerous reports have demonstrated the incidence and the patterns of resistance in many pathogens, few studies about the endemic antimicrobial resistance profile in developing countries were published. Hence, an evidence-based knowledge regarding the local antimicrobial resistance pattern is fundamental for guiding both antimicrobial treatment and empirical therapy of specific pathogens" [(13)]. "This guide is also important for effective antimicrobial stewardship as well as in the design of local and universal research programs" [(14)].

"P. aeruginosa synthesizes a secretory apparatus (Type III) that allows it to inject toxins from their cytoplasm into the target cell; T3SS is shared among many pathogenic Gram-negative bacteria. The latter mechanism allows mucoid bacteria to lyse the host's macrophages and overcome various defenses, such as with cystic fibrosis lung infection" [(15)].

"P. aeruginosa T3SS is a major determinant of virulence, and its expression is frequently associated with acute invasive infections and has been linked to increased mortality in

infected patients. The needle-like appendage of the T3SS, evolutionarily related to flagella, permits the translocation of effector proteins from the bacterium into the host cell through a spore formed in the host cell membrane. The exact contribution of each of the toxins to pathogenesis is unclear, but it is thought that the T3SS may allow *Pseudomonas* to exploit breaches in the epithelial barrier by antagonizing wound healing during colonization and to promote cell injury" [(16)].

"Multidrug resistant pathogen was defined as a pathogen that develops resistance to at least one agent in 3 or more antimicrobial categories. Other terms which are extensively used are extensive drug resistance (XDR) which is defined as a pathogen resistant to one agent in all categories except 2 or less; and Pandrug drug resistant (PDR) which is defined as a pathogen resistant to one agent in all categories" [(17)].

"The categories which classify the anti- *Pseudomonas aeruginosa* antimicrobial agents include Aminoglycoside (gentamicin, tobramycin, amikacin and netilmicin); Carbapenem (imipenem, meropenem and doripenem); Cephalosporins (ceftazidime and cefepime); Fluoroquinolones (ciprofloxacin and levofloxacin); Penicillins β-lactamase inhibitors (Ticarcillin-clavulanic acid and Piperacillin-tazobactam); Monobactam (aztreonam); Phosphonic acids (fosfomycin); Polymyxins (colistin and polymyxin B)" [(18)].

"Besides being intrinsically resistant to several antimicrobial agents, P. aeruginosa often acquires mechanisms of resistance to other antibiotics. Previous treatment with antibiotics that are characterized by high anti-pseudomonal activity and prolonged antibiotic treatment are both recognized risk factors for the emergence of drug resistant P. aeruginosa. Acquisition of strains resistant to ceftazidime, imipenem, piperacillin, or ciprofloxacin is associated with significantly longer hospital stays and an increased rate of secondary bacteremia in patients with P. aeruginosa infection. The increasing use of antibiotics and growing numbers of invasive procedures, together with the development of intrinsic and acquired resistance mechanisms of P. aeruginosa, causes the evolution of numerous multidrug resistant (MDR) P. aeruginosa outbreaks in clinical settings"[(30)]. The risk of acquiring MDR organisms may be related to the number of carriers in the same ward and to individual risk factors, such as patient characteristics and in-hospital events (invasive devices and antibiotic treatment).

"Resistance to antimicrobial agents is an increasing clinical problem and is a recognized public health threat. P. aeruginosa is one of the main organisms responsible for drug-resistant nosocomial infections and is a leading cause of bacteremia and nosocomial pneumonia. Multidrug Resistance P. aeruginosa is naturally resistant to a significant number of antimicrobials (Ampicillin, Amoxicillin, amoxicillin/clavulanate, first-generation cephalosporins, second-generation, cefotaxime, ceftriaxone, nalidixic acid). Furthermore, they easily acquire resistance to new antibacterial agents by mutational changes or by acquisition of genetic material. Emergence of MDR strains is often due to selective pressure of antimicrobial therapy. Genetic studies confirm the selection of resistant mutants and their subsequent spread" [(19)].

Antimicrobial resistance is rapidly becoming a global focus of attention, especially with the rising number of microorganisms resistant to available antimicrobials. Ps. aeruginosa is an extraordinary pathogen that can develop rapid resistance mechanisms to antimicrobial agents through chromosomal mutations or acquire extra-chromosomal materials from surrounding environments. Multi- and extensive-drugs resistant Ps. aeruginosa strains emerged worldwide and severely reduced treatment options.

The problem of multi-drug resistant microorganisms has arisen due to inappropriate use or discontinuous of antibiotics treatment, especially in Sudan, because most Sudanese take antibiotics without consulting a specialist. Therefore, identification of drug-resistant strains prior to antibiotics exposure is crucial to avoid treatment failure and prevent emerging drug resistance. This study aimed to identify drug-resistant patterns of Ps. aeruginosa strains in Khartoum Hospitals, Sudan.

Material and Methods:

A cross-sectional study was carrying out during the period from August to November 2021 at Royal care hospital, Alsilah Altiby hospital, Albaraha Hospital,

A total of 77 Pseudomonas aeruginosa isolated patients (33 males and 44 females) with age groups ranging from 10 to 60 years old. All patients were informed of the purpose of the study and their consent, or that of their care provider, was obtained before samples were collected.

Each patient was asked to collect approximately 10-20 ml of midstream urine into a sterile urine container, as for the ear and wound swab sample. A laboratory specialist collected it. After giving proper instructions to avoid contamination and samples were processed in the laboratory within 2 hours of collection. None of the patients admitted to consuming antibiotics during the 2 weeks prior to sample collection.

A structured questionnaire and referring to the patient clinical sheet were being used to collect demographic data and other data.

Isolation and identification of Pseudomonas aeruginosa using biochemical tests

Samples cultures were performed using semi-quantitative technique whereby samples were inoculated on cysteine-Lactose electrolyte deficient (CLED) medium plates at 37°C for 18-24 hours. Isolated colonies from significant plates were identified and differentiated from related organisms using standard conventional biochemical tests (Kligler Iron agar: slant /Alkaline, butt/ Alkaline, H2S no production / -, Gas / +; Motility test / motile; Indole /-, Urease /+; Citrate /+) according to [20]

Antimicrobial susceptibility testing

Antimicrobial sensitivity testing of all isolates was performed on diagnostic sensitivity test plates according to the Kirby-Bauer method [21] following the definition of the Committee of Clinical Laboratory International Standards [22]. Bacterial inoculums were prepared by suspending the freshly grown bacteria in 5mL sterile saline. A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. Filter paper disks containing a designated concentration of the antimicrobial drugs were obtained from Hi-Media Laboratories in the following concentrations: Cefotaxime, ceftriaxone, ciprofloxacin, ceftazidime, cefpodoime. The diameters of the zone of inhibition were interpreted according to CLSI standards. Media and disks were tested for quality control with Pseudomonas aeruginosa standard strain.

Data analysis

Statistical analysis was done by using the Statistical Package for Social Science program (version 20).

Results:

The present study used purposive sampling as the sampling technique**. Multidrug-Resistant *Pseudomonas aeruginosa* isolated from clinical specimens using the Kirby-Bauer Disk Diffusion Susceptibility Test-2021.**

The size of the sample was (77) Sample characteristics included, Gender and Age, etc.

The frequency and percentage of the samples are presented in the following tables:-

Socio-demographic Data:

The participants responders according to gender were female by 57.1%, while Male were 42.9%, table.1

Table (1): Distribution of participant according to the Gender:

{No=77}

		Frequency	Percent
Valid	Male	33	42.9
	Female	44	57.1
	Total	**77**	**100.0**

Distribution of participant according to the Age:

The participant's responders according to group age, the group between (21-35 y) were 50.6% and the group between (36-60y) were 42.9, while group between (10-20y) was 6.5%. table.2

Table (2): Distribution of participant according to the Age

{No=77}

		Frequency	Percent
Valid	10-20 y	5	6.5
	21 – 35 y	39	50.6
	36 – 60 y	33	42.9
	Total	**77**	**100.0**

Mean and Median of Age in the study group:

The table showed mean and median of age in the study group, the mean was 2.36 and the median was 2.00, (table.3)

Table (3): Mean and Median of Age in the study group

Age	
N	77
Mean	2.36
Median	2.00

Isolated Organism:

The table showed that the isolated organism test is *Pseudomonas aeruginosa* for all the participant's responders by 100% (Table.4)

Table (4): Isolated Organism

{No=77}

		Frequency	Percent
Valid	*Pseudomonas aeruginosa*	77	100.0

Frequencies of antibiotics sensitivity test:

Frequencies of Cefotaxime

The frequencies of **Cefotaxime** antibiotics for the Urine 22.6% (7/31) were sensitive, while 77.4% (24/31) was resistant., for the wound 41.2% (14/34) was sensitive, while 58.8% (20/34) was resistant., for the Ear 5.9% (2/12) was sensitive, while 29.4% (10/12) was resistant

Resistant average for **Cefotaxime** was 70.1%, and there is a high statistically significant relation between Cefotaxime antibiotics and samples (Urine and wound) and a normal relation with Ear, table (5).

P. value = (.000, .226)

Table (5): Frequencies of Cefotaxime antibiotics sensitivity:

(1) :Cefotaxime (CTX)		Frequency	Percent %	Resistant average	Correlations	P value
Urine	S	7	22.6	70.1%	1	.000
	R	24	77.4			
	Total	31	100.0			
wound	S	14	41.2		.595	.000
	R	20	58.8			
	Total	34	100.0			
Ear	S	2	5.9		.378	.226
	R	10	29.4			
	Total	12	35.3			

Frequencies of Ceftriaxone:

The frequencies of **Ceftriaxone** antibiotics for the Urine 26.5% (9/31) were sensitive, while 64.7% (22/31) was resistant., for the wound 29.4% (10/34) was sensitive, while 70.6% (24/34) was resistant., for the Ear 17.6% (6/12) was sensitive, while 17.6% (6/12) was resistant.

Resistant average for **Ceftriaxone** was 67.5%, and there is a high statistically significant relation between ceftriaxone antibiotics and samples (Urine & wound & Ear), table (6).

Table (6): Frequencies of Ceftriaxone antibiotics sensitivity:

(2): Ceftriaxone(CRO)		Frequency	Percent %	Resistant average	Correlations	P value
Urine	S	9	26.5	67.5%	1	.049
	R	22	64.7			
	Total	31	91.2			
Wound	S	10	29.4		.927	.000
	R	24	70.6			
	Total	34	100.0			
Ear	S	6	17.6		.557	.145
	R	6	17.6			
	Total	12	35.3			

Frequencies of Ceftazidime:

The frequencies of **Ceftazidime** antibiotics for the Urine 38.2% (13/31) were sensitive, while 52.9% (18/31) was resistant., for the wound 38.2% (13/34) was sensitive, while 61.8% (21/34) was resistant., for the Ear 20.6% (7/12) was sensitive, while 14.7% (5/12) was resistant.

Resistant average for **Ceftazidime** was 57.1%, and there is a high statistically significant relation between ceftazidime antibiotics and samples (Urine and wound) and a no relation with Ear, table (7).

P. value=.000

Table (7): Frequencies of Ceftazidime antibiotics sensitivity

(3): Ceftazidime (CAZ)		Frequency	Percent %	Resistant average	Correlations	P value
Urine	S	13	38.2	57.1%	1	.000
	R	18	52.9			
	Total	31	91.2			
Wound	S	13	38.2		1	.000
	R	21	61.8			
	Total	34	100.0			
Ear	S	7	20.6		-	.000
	R	5	14.7			
	Total	12	35.3			

Frequencies of Cefpodoxime:

The frequencies of **Cefpodoxime** antibiotics for the Urine 32.4% (11/31) was sensitive, while 58.8% (20/31) was resistant., for the wound 20.6% (7/34) was sensitive, while 79.4% (27/34) was resistant., for the Ear 5.9% (2/12) was sensitive, while 29.4% (10/12) was resistant.

Resistant average for **Cefpodoxime** was 74.0%, and there is a high statistically significant relation between cefpodoxime antibiotics and samples (Urine and wound) and a normal relation with Ear , table (8).

P. value= (.000, .226)

Table (8): Frequencies of Cefpodoxime antibiotics sensitivity

(4):Cefpodoxime (CPD)		Frequency	Percent %	Resistant average	Correlations	P value
Urine	S	11	32.4	74.0%	1	.000
	R	20	58.8			
	Total	31	91.2			
Wound	S	7	20.6		.728	.000
	R	27	79.4			
	Total	34	100.0			
Ear	S	2	5.9		.378	.226
	R	10	29.4			
	Total	12	35.3			

Frequencies of Aztreonam:

The frequencies of **Aztreonam** antibiotics for the Urine 55.9% (19/31) was sensitive, while 35.3% (12/31) was resistant., for the wound 35.3% (12/34) was sensitive, while 38.2% (13/34) was resistant., for the Ear 17.6% (6/12) was sensitive, while 17.6% (6/12) was resistant.

Resistant average for **Aztreonam** was 40.2%. table (9).

P. value= (.000, .005)

Table (9): Frequencies of Aztreonam antibiotics sensitivity

(5):Aztreonam (ATM)		Frequency	Percent %	Resistant average	Correlations	P value
Urine	S	19	55.9	40.2%	1	.000
	R	12	35.3			
	Total	31	91.2			
Wound	S	12	35.3		.540	.005
	R	13	38.2			
	Total	25	73.5			
Ear	S	6	17.6		-	.000
	R	6	17.6			
	Total	12	35.3			

Frequencies of Meropenem:

The frequencies of **Meropenem** antibiotics for the Urine 19% (25/31) was sensitive, while 17.6% (6/31) was resistant., for the wound 79.4% (27/34) was sensitive, while 20.6% (7/34) was resistant., for the Ear 26.5% (9/12) was sensitive, while 8.8% (3/12) was resistant, table (10).
Resistant average for **Meropenem** was 5.33% .**P. value= .000**

Table (10): Frequencies of Meropenem antibiotics sensitivity

(6):Meropenem (MEM)		Frequency	Percent %	Resistant average	Correlations	P value
Urine	S	25	19%			
	R	6	17.6		1	.000
	Total	31	91.2			
Wound	S	27	79.4			
	R	7	20.6	20.7%	.786**	.000
	Total	34	100.0			
Ear	S	9	26.5			
	R	3	8.8		-	.000
	Total	12	35.3			

Discussion:

Antimicrobial resistance is rapidly becoming a global focus of attention, especially with the rising number of microorganisms resistant to available antimicrobials. Ps. aeruginosa is an extraordinary pathogen that can develop rapid resistance mechanisms to antimicrobial agents through chromosomal mutations or acquire extra-chromosomal materials from surrounding environments. The aim of the present study was to determine the multi-drug resistant pattern of Pseudomonas aeruginosa isolated from clinical specimens.

The results revealed that the majority they were responders off from Female by 57.1%, while Male were 42.9%, and the age Most from between (21-35 y) was 50.6% and the between (36-60y) were 42.9 and the isolated organism test is Pseudomonas aeruginosa for all the participant's responders by 100%

The results revealed that the frequencies of Cefotaxime antibiotics for 7 urine samples were sensitive by 22.6% and 24 was resistant by 77.4%, for the wound samples 14 sensitive by 41.2% and 20 resistant by 58.8%, for the Ear samples 2 were sensitive by 5.9% and 10 was resistant by 29.4%.

"Therefore, the frequency of Ceftriaxone antibiotics for 9 urine samples were sensitive by 26.5% and 22 were resistant by 64.7%, frequency of Ceftazidime antibiotic for 13 urine samples were sensitive by 38.2% and 18 were resistant by 52.9% and frequency of Aztreonam antibiotics for 19 urine samples were sensitive by 55.9% and 12 were resistant by

35.3%, increasing resistance of beta-lactam antimicrobial in nosocomial Aeruginosa has become a serious threat particularly against third and fourth-generation Cephalosporin's. There are many molecular mechanisms to develop resistance against these antibiotics; generation of extended spectrum beta-lactamases (ESBL) by incorporation of bla genes in integrons and inability of porin genes to enhance their expression level and/or alteration of antibiotic target sites" [23].

Nevertheless, this study showed a high resistant rate in cephalosporin family third generation, (cefotaxime 70%, ceftriaxone 67%, ceftazidime 57%, cefpodoxime74%, and monobactam antibiotic aztreonam 40%, with less resistant percentage in meropenem20%) the result is similar to Zaheer Ali study in Karachi (2019) [24] and Romika Dawra study in India (2017) [38] and draw a conclusion about the highly resistant of cephalosporin third generation and the most effective antibiotic was meropenem.

The most interesting side is study in Nepal (2018) [24] by S Upadhaya and show the most aggressive resistance in meropenem (94.1%) and cefotaxime (76.5%) and the result disagrees as the meropenem is the drug of choice.

It's highly recommended to focus on drug resistance mechanisms for all microorganisms especially MDR P. aeruginosa to eradicate infection and superbugs [25], restriction of using antimicrobial agents without physician presubscription and control of infection is highly recommended to prevent increasing MDR Pa population in community (26) .

There is an urgent need to resolve the issue by taking some preventive measures. Combined efforts of health care professionals and researchers are required to educate people about the proper use of antibiotics and other infection control measures.

Conclusion:

Drug resistance in *P. aeruginosa* is a multifactorial increasing phenomenon. Estimated frequencies were between 12-36%. Mechanisms of resistance either through membrane permeability and efflux system or through its virulence factors or acquired genetically by plasmid. Combination of these mechanisms leads to a superbug, which is very difficult to be treated.

Ethical Approval:

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

Consent

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

Disclaimer (Artificial intelligence)
Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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