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Evaluation Effect Of Amikacin-Cefepime Combination Against Biofilm Formation In XDR-*Pseudomonas.aeruginosa*

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ABSTRACT

Background: *The intent of the research was to evaluate how the combination of amikacin and cefepim affected biofilm formation in ten clinical isolates of extensive druge resist pseudomonas aeruginosa A total of 295 clinical samples were obtained from patients suffering from burns, UTI , wounds, and CSF. Laboratory diagnosis is based on biochemical and morphological testing, and the VITEK-2 Compact system is used for confirmation. 53 Pseudomonas aeruginosa isolates from 295 samples were identified in the findings, representing 18% of the total samples . Susceptibility test showed that isolates had greater percentages of resistance to ceftazidime (72%)followed by cefepime (60%) , while has low resist to colstin (2%) . 35 isolates (66%) of Pseudomonas aeruginosa were detected as multi druge resist , ten (19 %) bacterial isolates resist more than five classes of antibiotics which considered As extensive druge resistance (XDR) . All XDR pseudomonas .aeruginosa showing strong biofilm formation .Combinations of cefepime and amikacin are more effective than their individual at preventing P. aeruginosa from forming biofilms in an in vitro environment .*

Keywords: *Biofilm , Laboratory diagnosis, Extensive druge resistance.*

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INTRODUCTION

Millions of individuals throughout the world suffer from infectious illnesses brought on by bacteria and fungus. Different harmful organisms respond differently to different antibiotics' inhibitory effects, therefore the emergence as well as dissemination of antibacterial resistance is a global problematic. The indiscriminate besides incorrect use of existing antimicrobial medications is blamed for the rising problem of bacteria developing resistance to antimicrobial treatments (Brown and Wright., 2016). Clinically significant microorganisms have numerous antibiotic resistances in addition to a single drug resistance (Horcajada *et al*., 2019).

Opportunistic bacteria *Pseudomonas aeruginosa* may cause infections In people with compromised immune system, utmost frequently among individuals receiving treatment in surgical, burn, and acute care units. The respiratory system, placenta, urinary system, skin, and soft tissues are the most often infected organs by P. aeruginosa; contamination of surgical incisions, burns, and pressure

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ulcers poses the highest risk (Pachori *et al.*, 2019). It is among the top five pathogens that cause healthcare-associated infections (HAI) (Askoura *et al.*, 2011). Biofilms give *P. aeruginosa* a considerable advantage by facilitating life on synthetic materials, immune system evasion, and resistance to antibiotic treatment. (Maurice, Bedi, and Sadikot 2018). One crucial aspect that enables *P. aeruginosa* to generate severe and persistent infections associated with considerable morbidity and death, for instance, is its capacity to build biofilms. *Pseudomonas.aeruginosa* biofilms significantly increase the risk of mortality among individuals having cystic fibrosis (CF)(Mulcahy et al., 2014). Numerous studies have been conducted to determine how this bacterium species forms biofilms and how different surface types affect bacterial adherence and biofilm development (Mouhamed *et al.*, 2014). Previous research has shown a correlation between P. aeruginosa's capacity to produce biofilms and its distinctive feature of resistance to many drugs (Yekani *et al.*, 2017).

The management of biofilm infection remains difficult today. Traditional antibiotics need to be present in very high doses in order to to eradicate bacteria in test tube biofilms being formed; however, this is challenging to do in vivo *(*Shortridge et al ., 2019). Cefepim is the main agent in a combination that targets the non growing biofilm population that is resistant to the majority of other antibiotics. FEP, however, has little impact on the biofilm subgroup that is actively expanding. Consequently, in order for the combined utilization of antibiotics to be effective, they must be able to wipe out the active FEP-resistant cap population and break through the biofilm. The purpose of this research is to examine the efficacy of combining medicines (amikacin+ cefepime) in which the XDR-*p.aeruginosa* is already resistant on biofilm formation .

METHODS

Clinical samples from patient sources were obtained throughout the monitoring period of January, February, and March 2023. Al-Sadder Medical City, AL-Najaf Hospital , and Burn Center . Including urine samples, wound exudate, burn exudate, ear swab , and CSF aspirate . Isolates were identified as *pseudomonas.aeruginosa* by biochemical tests (urease, simmon citrate, indole, oxidase and catalase), selective media (MacConkey agar, Cetrimide agar) and confirmed the identification using Vitek II. isolates of *P. aeruginosa* were tested for antibiotic susceptibility using 11 antibiotics, which are represented by Piperacillin (PRL), Ceftazidime (CAZ), Cefepime(FEP), Aztreonam (ATM), (CEP), Imipenem (IMP), Meropenem (MEM), Amikacin (AK), Tobramycin (TOB),Ciprofloxacin (CIP), and Levofloxacin (LEV) with the use of the disc diffusion method All of them, with the exception of Colistin (CN), which was identified by use of the MIC method. the results were compared to the National Community for Clinical Laboratory Standard (CLSI, 2023) results. biofilm development by P. aeruginosa was evaluated Microtiter plate method using ELISA auto reader as defined by (Patel et al. 2016) . Utilizing a 96-well microtiter plate, the antibiofilm activity of cefepime and amikacin was assessed each one alone and in combination.

RESULTS

A total of 295samples from patient sources were obtained throughout this study 53 isolates of *P. aeruginosa* samples were originally identified using conventional technique and it confirmed with VITEK-2 system . On blood agar, *P. aeruginosa* often exhibits beta hemolysis, a metallic sheen, and a green

pigment, While on MacConkey agar, lactose did not ferment, resulting in small pale colonies. When placed on cetrimide agar, the agar appears mucoid, smooth, with raised centers and flat borders, and has a fruity odor as shown in figure 1.

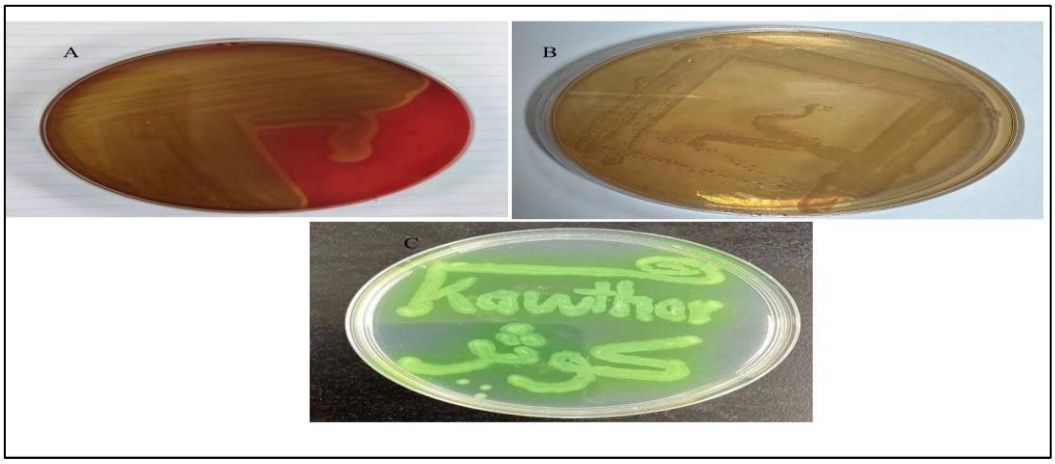

Figure 1. Colonies of *P. aeruginosa* on (A) blood agar (B) MacConkey Agar (C) on Citrimde Agar following a 24-hour incubation at 37˚C.

In the current investigation, isolates of *P. aeruginosa* were tested for 11 antibiotic ,Sensitivity test showed that isolates had greater percentages of resistance to ceftazidime (72%)followed by cefepime (60%) , while has low resist to colstin (2%) as indicate in figure .3

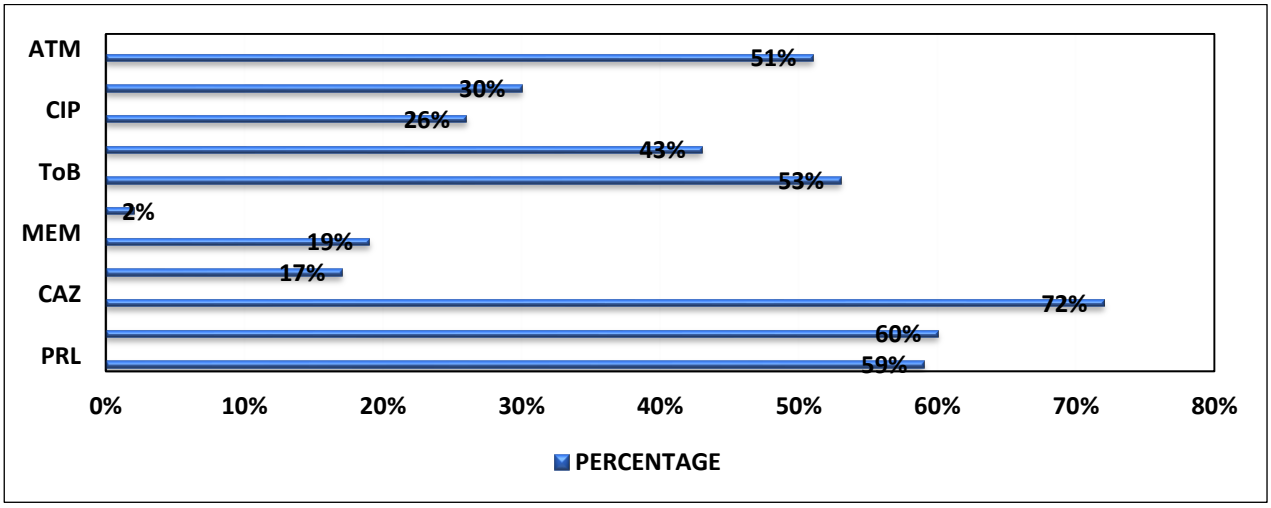

Figure 3. Antibiotic Resistance Percentage of *Pseudomonas aeruginosa* isolates . (*P*): *P. aeruginosa*, (PRL): Piperacillin **, (FEP):** Cefepime**, (CAZ) :** Ceftazidime, **(IMP):** Imipenem **(MEM):**Meropenem **,** (**TOB):** Tobramycin**,** (**AK):** Amikacin**,** (**CIP):** Ciprofloxacin**,** (**LEV):** Levofloxacin **,** (**CN):** colsin **, (ATM):** Azterionam **.**

Ten *P. aeruginosa* isolates that were consider as extensive -drug resistant were used in the current investigation, as indicated in Table .1

Table 1.Percentage Resistance of XDR -*pseudomonas .aeruginosa*

No.	Sources of isolates	Percentage of resistance
P1	**Burn**	**100%**
P2	**Burn**	**90%**
P3	**Burn**	**90%**
P4	**Ear swab**	**90%**
P5	**Burn**	**81%**
P6	**Burn**	**81%**
P7	**Burn**	**72%**
P8	**Burn**	**72%**
P9	**Burn**	**72%**
P10	**Urine**	**72%**

XDR-isolates that used in this exprement showing highest resistance to amikacin and cefepime as indicated in the table 2.where discovered that the MIC, or minimum inhibitory concentration, was calculated .

Table 2. MIC amikacin and cefepime values against ten *P.aeruginosa* .

Isolate	Amikacin (mcg/ml)	Cefepime (mcg/ml)
	MIC	**MIC**
P1	128	512
P2	64	512
P3	128	512
P4	128	256
P5	256	1024
P6	256	1024
P7	128	1024
P8	128	1024
P9	128	1024
P10	128	256

in this study, a positive relationship could be seen between the production of biofilm and resistance to antibiotics in the *P. aeruginosa* isolates. All XDR *pseudomonas .aeruginosa* showing strong biofilm formation as indicate in the table 3.

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Table 3. Biofilm formation of *P. aeruginosa*

Clinical isolate	Source of isolate	Biofilm production
P_1	Burn	Strong
P_2	Burn	Strong
P_3	Burn	Strong
P_4	Ear swab	Strong
P_5	Burn	Strong
P_6	Burn	Strong
P_7	Burn	Strong
P_8	Burn	Strong
P_9	Burn	Strong
P_{10}	Urine	Strong

This study find ihibiting biofilm formation whene treated with cefepime and amikacin sepertly alone with high concentration as shows in table 5,6. and ihibit biofilm formation whene treated with amikacin-cefepime combination in low concentration as indicated in table 7.

Table 5. *P. aeruginosa* isolates' biofilm development both before and after amikacin therapy.

Isolates	Before treatment	After treatment with amikacin Concentration (mcg/ml)							
		4	8	16	32	64	128	256	512
P1	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P2	Strong	Strong	Strong	Moderate	No biofilm	No biofilm	No biofilm	No biofilm	No biofilm
P3	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P4	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P5	Strong	Strong	Strong	Strong	Moderate	Weak	No biofilm	No biofilm	No biofilm
P6	Strong	Strong	Strong	Strong	Moderate	Weak	No biofilm	No biofilm	No biofilm
P7	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P8	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P9	Strong	Strong	Strong	Moderate	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P10	Strong	Strong	Strong	Moderate	Weak	No biofilm	No biofilm	No biofilm	No biofilm

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Table 6. *P. aeruginosa* isolates' biofilm development both before and after cefepime therapy.

Isolates	Before treatment	After treatment with cefepim Concentration (mcg/ml)							
		8	16	32	64	128	256	512	1024
P1	Strong	Strong	Strong	Strong	Strong	Moderate	No biofilm	No biofilm	No biofilm
P2	Strong	Strong	Strong	Strong	Strong	Moderate	No biofilm	No biofilm	No biofilm
P3	Strong	Strong	Strong	Strong	Moderate	Moderate	Weak	No biofilm	No biofilm
P4	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P5	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P6	Strong	Strong	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm
P7	Strong	Strong	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm
P8	Strong	Strong	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm
P9	Strong	Strong	Strong	Moderate	Moderate	Weak	No biofilm	No biofilm	No biofilm
P10	Strong	Strong	Strong	Moderate	Weak	No biofilm	No biofilm	No biofilm	No biofilm

Table 7. P. aeruginosa isolates' biofilm development both before and after combination of (cefepime / amikacin)therapy.

Isolates	Before treatment	After treatment with cefepim /amikacin combination Concentration (mcg/ml)							
		1/0.5	2/1	4/2	8/4	16/8	32/16	64/32	128/64
P1	Strong	Strong	Strong	Weak	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P2	Strong	Strong	Strong	Moderate	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P3	Strong	Strong	Moderate	Moderate	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P4	Strong	Strong	Strong	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm
P5	Strong	Weak	No biofilm	No biofilm	No biofilm	No biofilm	No biofilm	No biofilm	No biofilm
P6	Strong	Strong	Strong	Strong	No biofilm	No biofilm	No biofilm	No biofilm	No biofilm
P7	Strong	Strong	Strong	Moderate	Moderate	No biofilm	N0 biofilm	No biofilm	No biofilm
P8	Strong	Strong	Strong	Moderate	Moderate	No biofilm	No biofilm	No biofilm	No biofilm
P9	Strong	Strong	Strong	Moderate	Moderate	No biofilm	No biofilm	No biofilm	No biofilm
P10	Strong	Strong	Strong	Moderate	Moderate	No biofilm	No biofilm	No biofilm	No biofilm

DISCUSSION

Numerous research examined the capacity of clinical isolates derived from hospitals to produce a substantial amount of biofilm. (Zgair et al., 2013; Wojtyczka et al., 2014). Thus, in this study, a positive relationship could be seen between antibiotic

resistance and biofilm formation in the *P. aeruginosa* isolates.

The management of biofilm infection remains difficult today , treatment for infections associated with biofilms might occasionally be difficult due to multidrug resistance (AlDahmoshi et al., 2020). In the current investigation, we found that to in vitro kill the live bacteria in the immature biofilm, we needed at least 64 to 128 mcg/ml to reduce biofilm formation in *pseudomonas.aeruginosa*, and 500 to 1000 mcg/ml FEP to eliminating biofilm , however we required at least 2000 mcg/ml FEP, This above the CLSI breakpoint by 60 times Such high quantities cannot be reached in living organisms, largely because kidney toxicity (Baghdasaryan and Nalbandian et al., 2023). The findings of this study demonstrated that the amikacin-cefepime combination prevented 80% and 100% of the biofilm development of *P. aeruginosa* at 8-16 mcg/ml.

CONCLUSION

In conclusion, all XDR *pseudomonas.aeruginosa* has stronge biofilm development .

Biofilm was hard to reduced by using antibiotic (amikacinand cefepime) indivisually , its reduced with high concentration of antibiotic , in another hand Combinations of cefepime and amikacin are more effective than their individual at preventing *P. aeruginosa* from forming biofilms in an in vitro environment .this combination can reduced biofilm completely with with low concentration .another combination shoud make to test against this bacteria .

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