ORIGINAL ARTICLE

Role of Biofilm from *Pseudomonas aeruginosa* in ocular infection in Baghdad

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ABSTRACT

Aim: To study *Pseudomonas aeruginosa* antibiotic sensitivity, resistance, and the biofilm capacity from patients that have contact-lens-associated diseases.
Methods: Total 159 specimens somewhat from four central hospitals within Baghdad City collected from infection which associated with eyes through January 2018 to July 2018, 115 of such specimens reported here that presence of bacteria had been positive. Amongst these, 42 (36.5%) seemed to be positive whereas 73 (63.5%) constituted negative gram stain. *Pseudomonas aeruginosa* 54 (46.9%), *Staphylococcus* spp. 25 (21.7%), *Acinetobacter* spp. 12 (10.5%), *Streptococcus* spp. 9(7.8%), *Micrococcus* spp. 7 (6.2%), *Serratia marcesens* 4 (3.5%), *E coli* 3 (2.6%) and *Bacillus* spp. 1 (0.8%).
Results: The resistance to antibiotics was for Ciprofloxacin (90.7%), Cefazoline (88.8%), Ofloxacin (87.03%), Gentamcin (83.3%), Cefepime (77.7%) as well as towards Ceftriaxone (75.9%) were recorded. High imipenem resistance (64,8%) has been followed by neomycin (62,9%), ampicillin – Sulbactam also Nitrofurantoin (61,1%) as well as ceftazidime (53,7%), and less resistance towards Tobromycin (33.3 %), and 38.8% toward Amikacin. Capacity with biofilm formation had also been observed throughout 96.3% and 3.7% of non-biofilm development.
Conclusions: Scientific understanding about biofilm development as well as resistance to antibiotics contribute to the discovery of other innovative goals toward Pseudomonas eye infection therapy. These typically continue to exist even after its long-term usage of different antibiotic treatment. Its capacity about progress throughout a biofilm improves their own opportunities of protecting themselves against host protection mechanisms, antimicrobial remedy or otherwise biocidal products.
Keywords: Biofilm, *Pseudomonas aeruginosa*, ocular infection, Baghdad

INTRODUCTION

Microbial contaminants relating to contact lenses were generally attached linked with eye infectious diseases, and they are often is related to bacterial, fungal, viral and other disease-related infections. Bacterial keratitis seems to be the generality serious type of such illnesses[1].

Keratitis become a severe eye disorder which may contribute greatly even more to corneal ulcers when kept unresolved either handled by insufficient drugs. Various factors, which including eye injury, superficial eye defects or corneal operations, may cause keratitis[2].

A further predisposition here to advancement with keratitis through healthy eyes includes the use of contact lenses. 60–70% about the contact lens-linked eye illnesses are associated with *P. aeruginosa*[3,4]. The principal reasons of such lenses illness can be assumed being the biofilm formed via *P. aeruginosa*. The crucial causal factors behind the infectivity of Pseudomonas infectious diseases are indeed bacterial surface stimuli, flagella, pili, lipopolysaccharide, and otherwise efficient activities which including toxin separation, biofilm formalization, quorum sensing[5]. Its development of biofilm becomes an important infectivity feature throughout the persistent of diseases.

The fact that biofilm has considerable medical implications becomes exceedingly clear[8]. Accordingly, considerable awareness has been devoted toward the contribution of biofilms in infection control. Biofilms even have phenotypical characteristics that behind its drug resistance throughout lens-contact infection [(6)]. Such resistance occurs because the pathogenic bacteria assembled across an exopolysaccharide matrix (EPS) that compose biofilm. Unsurprisingly, a massive increase about MDRs has rendered an option of effective management to infections hard. Multi - drug resistant *P. aeruginosa* pathogens have been, due to many reports, highly resistant towards a minimum of 3 groups of antibiotic drugs. Aminoglycosides, penicillin, carbapenems, cephalosporin and Quinolones are included. Frequent drug resistance investigation may further facilitate health care professionals select the right antibiotic [(7, 8)].

Within this analysis, the study of *Pseudomonas aeruginosa* antibiotic sensitivity / resistance status as well as the biofilm capacity from patients has contact-lens-associated diseases, also the exploration of the link between both the possible biofilm formation by Pseudomonas aeruginosa as well as the drug resistance status.

MATERIALS AND METHODS

Patients: 159 specimens were obtained, ranging (102 males as well as 57 females), of which ophthalmologists had been recognized them with eye infections. Throughout the time frame 3 January 2018 as well to 15 Jul 2018, cases are reported at four hospitals in Baghdad city.

Collection of samples: Exterior ocular surface eyes samples have been taken with cotton swabs as well as corneal scrap. The above samples had all been immediately injected on just transportation particular medium plates and after that incubated up overnight at 37 ˚C The background about every case had been documented with a survey[9].

***P. aeruginosa* identity / Morphological investigation:** Colonies growing throughout selective medium had been moreover distinguished by analyzing of own morphology, starting with Gram stain as well as manifestation under

microscopic examination (Gram response, morphology, configuration) [10].

Eye test samples cultivation: Three cultural media, including Blood agar, MacConkey agar as well as Cetrimide agar, had been used for inoculate gathered samples[11].

Checking of biochemical: Various biochemical assays, including oxidase test, catalase test, Indole production testing, methyl red testing, voge-proscaur test, Simmon citrate usage test, have been carried out for the identified colonies. Development around 42 °C, development around 4 °C, only *p. aeuroginosa* strains such possess the above capability would be used to classify isolates.

Susceptibility screening for antibacterial drugs: According to[12], Those specimens had been screened utilizing therapeutically essential antibacterial drugs such as Amoxicillin, Cefotaxime, Imipenem, Ciprofloxacin, Gentamicin, Cefepime, Cefozopran concerning antimicrobial sensibility via an agar disc diffusion procedure.afterward 18 hrs, each diameter about the inhibitory activity region was evaluated by comparing to *the Escherichia coli* ATCC 352218 monitoring strains.

Monitor and evaluate Biofilm Formation: Biofilm tests had been developed that can be seen in 2004 through Caiazza and O'Toole [13] using glucose here as supplemental for M63 media with CAA and also magnesium sulfate $MgSO_4$.

RESULTS AND DISCUSSION

Sum with 159 cases been determined at an ibn-Hiatham Eye training hospital in Baghdad regarding sufferers with keratitis around January (2018) till July (2018).

Isolation of bacteria: Including its 159 cases reported with ulcers, 115(72.32%) appeared positive in connection with bacteria following replication through MacConkey agar as well as blood agar. other remaining 44(27.68%) proved negative regardless of their continuation of incubation time even though no bacterial growth was recorded.

Identification of bacterial isolates: Following a microscopic, cultural as well as biochemical study, the findings of the suspicious isolated bacteria appear to show including of 115 pathogenic microorganisms' isolates, 42(64.76%) had been gram - positive bacteria; that include 25(21.7%) *Staphylococcus* spp., 9(7.8%) *Streptococcus* ssp., 7(6.2%) *Micrococcus* spp., as well as 1(0. 8%) *Bacillus* spp. Even as gram-negative bacteria, 73 pathogenic organisms, 54(46.9%) *Pseudomonas aeruginosa*., 12(10.5%). *Acinetobacter* spp., 4(3.5%) *Serratia marcesens* as well as 3(2.6%) *E. coli*. The pervasiveness about microbial keratitis-responsive microbes vary through place as well as time[14].

It had been assessed that MK positive culture would have been around 64.76%[19]. Here on opposite hand, an even more research demonstrates that just 40% about cases have positive culture. Those findings vary with numerous considerations which including samples size, attachment by microbes towards hard objects (intra - ocular lens, lens fragment), Therefore as result, cells decreased with vitreous aquatic humour, antimicrobial agents expected to take prior to the gathering even of therapeutic substance or even those triggering endophthalmitis mentioned throughout fastidious micro-organisms[15].

Researchers shown that More than 50(53.84%) in patients with bacterial isolated strains had been identified for *pseudomonas* spp.,Around 13(20%) about cases, *Staphylococcus* has been detected, and other study noted that Pseudomonas aeruginosa the most common bacteria casing corneal ulcer[16].

Above mentioned findings appear close towards the findings of ours, the primary isolation between many clinical pathogens both in Ghana and as well as southern India had already been noticed for being *Pseudomonas* spp. *Staphylococcus aureus* was, throughout another research, its dominant widespread isolated bacteria, however researchers linked the explanation of variability within the bacterial causes for keratitis towards numerous climatic situations, socioeconomic factors, nature & operation throughout those regions[17,18].

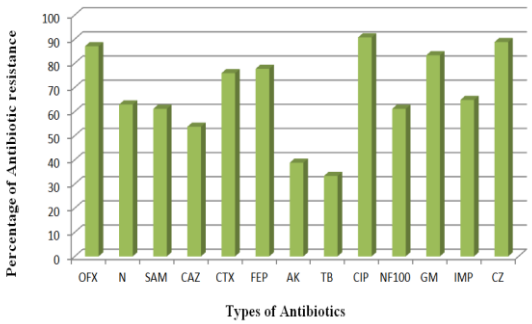
Table 1 show the percent of isolated bacteria from different types of patients suffers from eye infection .

Table 1: The Type and percentage of isolates according vitek-2 system results

Bacteria	No.	%age
Pseudomonas aeruginosa.	54	46.9%
Acinetobacter spp	12	10.5%
Serratia marcescens	4	3.5%
E.coli.	3	2.6%
Staphylococcus spp.	25	21.7%
Streptococcus spp.	9	7.8%
Micrococcus spp.	7	6.2%
Bacillus spp	1	0.8%
Total	115	100%

The *P. aeruginosa* isolates' antimicrobial sensitivity profiles can be seen in figure 1. Highly resistant had been detected to Ciprofloxacin (90.7%), Cefazolin (88.8%), Ofloxacin (87.0%), Gentamycin (83.3%), Cefepime (77.7%) as well as Ceftriaxone (75.9%). Modest Imipenem resistance (64.8%) had also been followed by Neomycin (62.9%), Ampicillin – Sulbactam as well as Nitrofurantoin (61.1%) and even Ceftazidamin (53%). Around the same time, Tobramycin (33.3 percent) and Amikacin (38.8 percent) were shown to have a limited degree of resistance and even the minimum antibiotic degree. MDR has been reported through all *P.aeruginosa* isolated bacteria.

Figure 1: *P. aeruginosa* isolates resistance towards antibiotics



A resistance status assessment about our own *P. aeruginosa* isolates collected that almost all of them are reluctant against one or however many antibiotic products which had been checked. Aminoglycosides just like Amikacin have been reported for being efficacious throughout *P.aeruginosa* induced eye problems, further, earlier research showed which treating P. aeruginosa infection with Amikacin seems to be successful. A recent research noticed that *P. aeruginosa* resistant to β-lactam as well as Fluoroquinolones classes of antibiotic[19].

Our results of the study agreed with those kind of publications, which showed least resistance to amikacin (aminoglycoside) as well as high resistance also to β-lactam and even fluoroquinolones throughout pseudomonas strains. Besides the previously mentioned research, moreover, high degree of resistance towards Gentamycin (aminoglycosides) has been reported. An even more researchers reported data that had been contradictory towards our findings, which showed that *Pseudomonas* had poor levels of resistance for fluoroquinolones like ofloxacin[20].

A specific technique shown during 2004 by Caiazza and O'Toole has been followed for the identification of the development with biofilms[13]. The strain progress as well as its separation at 37 ° C were evaluated, also soak up more than bacterial control group with (negative control) as well as provide its level including the shade of blue from every isolate table 2.

Table 2: Biofilm forming capacity of *P. aeruginosa* isolates

Production of *P. aeruginosa*	**%age**	**No of isolates**
Strong	22.2%	12
Moderate	31.5%	17
Weakly	42.6%	23
No formation biofilm	3.7%	2
Total	100%	54

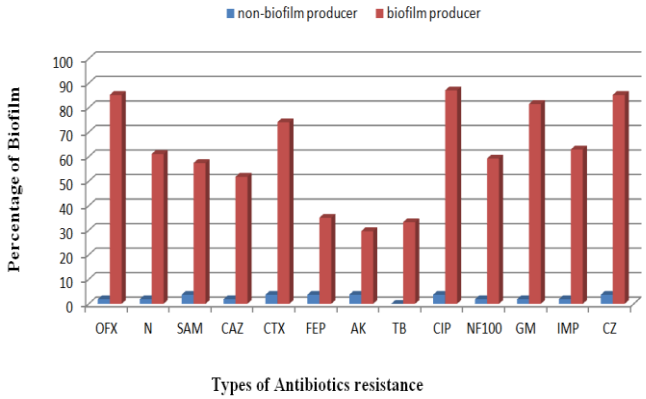
The quantitative methodology assessment of the opportunities for biofilm development reported that approximately *Pseudomonas aeruginosa* isolates were guiding biofilm formers[5]. The inappropriate treating or even usage of unsafe storing solutions may cause pollution to contact lens, which may in role serves as appropriate place to biofilm formation as well as microbial adherence. Improved resistance to antibiotics, eventually leading to therapeutically disappointment, had already linked the power to form up biofilms[21]. Through our analysis it was found whether Pseudomonas aeruginosa that biofilm formers possess the characteristic of antibiotic resistance, whereas non-biofilm creators were the isolates that are minimal resistant to the antibiotic, by which the MIC of various antibiotics have been believed to improve about 10-1000 times within particular bacterial biofilm formation while opposed to non-biofilm producers[22].

Three different techniques may even describe the above tolerance:

1. The inability by antibacterial drugs to reach through thick matrix,
2. Suboptimum antibiotic level, when such an antibiotic infiltrate into the biofilm, under minimum inhibition levels towards microbes within the biofilm,
3. The antimicrobial agent cannot kill pathogenic bacteria when many of the bacteria become metabolically inert throughout deeper forms with biofilm, As well as (4) Removal of antibiotics out of a biofilm via bacterial populations mostly as general rule with combined "efflux action"[23].

Microorganisms which including Pseudomonas aeruginosa seem to be lenses washing suspension resistant via adhering as well as extending through establishment domain of lipids[24,25].

Figure 2: The relationship between antibiotic- resistance and biofilm formation



CONCLUSIONS

The involvement of risks is towards both Gram positive as well as Gram negative pathogens through the tissues about ophthalmic, furthermore, the significant source causing ocular infections being gram - negative bacterium. Biofilm is essential for enhancing pathogenic potential within bacteria.

Source of Funding: Self funding
Conflict of Interest: No conflict of interest

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Supplement 1: Types of Biofilm about *P. aeruginosa*

No.	Isolates	Absorption	Types of Biofilm	No.	Isolates	Absorption	Types of Biofilm
1	1	1.03	High	29	45	0.2	Mediate
2	3	0.954	High	30	23	0.187	Weak
3	20	0.676	High	31	31	0.182	Weak
4	4	0.585	High	32	29	0.176	Weak
5	26	0.563	High	33	50	0.167	Weak
6	5	0.552	High	34	42	0.164	Weak
7	33	0.505	High	35	54	0.159	Weak
8	28	0.484	High	36	10	0.157	Weak
9	11	0.458	High	37	30	0.155	Weak
10	36	0.436	High	38	27	0.144	Weak
11	7	0.433	High	39	19	0.139	Weak
12	8	0.426	High	40	25	0.134	Weak
13	2	0.398	Mediate	41	46	0.133	Weak
14	48	0.395	Mediate	42	14	0.128	Weak
15	6	0.389	Mediate	43	12	0.125	Weak
16	13	0.365	Mediate	44	21	0.123	Weak
17	9	0.358	Mediate	45	41	0.121	Weak
18	52	0.342	Mediate	46	44	0.117	Weak
19	49	0.311	Mediate	47	34	0.116	Weak
20	15	0.288	Mediate	48	17	0.114	Weak
21	53	0.273	Mediate	49	40	0.11	Weak
22	37	0.268	Mediate	50	16	0.108	Weak
23	24	0.263	Mediate	51	43	0.104	Weak
24	38	0.244	Mediate	52	35	0.102	Weak
25	22	0.228	Mediate	53	18	0.099	Non
26	32	0.208	Mediate	54	47	0.0997	Non
27	51	0.206	Mediate		cutt off	0.1	
28	39	0.203	Mediate				

Supplement 2: Results of antibiotics resistances of *P. aeruginosa*

Isolates	OFX	N	SAM	CAZ	CTX	FEP	AK	TB	CIP	NF100	GM	IMP	CZ
PS.1	**R**	**R**	**R**	**R**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**R**
PS.2	**R**	**I**	**R**	**S**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	R	**R**
PS.3	**R**	**S**	**S**	**R**	**R**	**R**	**S**	**I**	**R**	**R**	**R**	S	**R**
PS.4	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	R	**R**
PS.5	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	S	**R**
PS.6	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	R	**R**
PS.7	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**S**	**R**	**R**	**I**	R	**R**
PS.8	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	S	**R**
PS.9	**R**	**I**	**S**	**R**	**R**	**S**	**S**	**I**	**R**	**R**	**R**	S	S
PS.10	**R**	**R**	**R**	**R**	**R**	**I**	**S**	**S**	**R**	**R**	**R**	R	R
PS.11	**R**	**R**	**R**	**R**	**R**	**S**	**I**	**S**	R	**R**	R	R	R
PS.12	**I**	**R**	**I**	**R**	**I**	**R**	**R**	**I**	R	**R**	R	R	R
PS.13	**R**	**I**	**R**	**S**	**S**	**R**	R	R	R	S	R	R	R
PS.14	**R**	**R**	**R**	**R**	R	**R**	**S**	**I**	R	**R**	R	**S**	R
PS.15	**R**	**R**	**R**	**S**	R	**I**	**R**	**S**	R	**S**	**S**	**R**	R
PS.16	**R**	**S**	**S**	**R**	R	R	**R**	**S**	R	**I**	**R**	**S**	R
PS.17	**R**	**R**	**R**	**R**	R	R	**S**	**R**	R	**R**	**R**	**R**	R
PS.18	**R**	**S**	**R**	**R**	R	R	**R**	**S**	R	**S**	**S**	**I**	S
PS.19	**R**	**R**	**S**	**S**	R	R	**I**	**R**	R	**R**	**R**	**S**	R
PS.20	**R**	**R**	**R**	**S**	R	R	**R**	**I**	R	**R**	**R**	**R**	S
PS.21	R	R	**S**	**S**	**R**	R	**S**	**I**	R	**R**	R	**R**	R
PS.22	R	R	**S**	**R**	**R**	R	**S**	**I**	R	**R**	R	**R**	R
PS.23	I	R	**R**	**I**	**S**	R	**S**	**R**	R	**S**	R	**S**	R
PS.24	R	I	**R**	**S**	**R**	**I**	**S**	**R**	R	**S**	**S**	**R**	R
PS.25	R	S	**S**	**R**	**R**	**R**	**S**	**I**	R	**R**	**R**	**S**	R
PS.26	R	R	**S**	**S**	**R**	**I**	**R**	**R**	R	**S**	**S**	**R**	R
PS.27	R	I	S	R	R	R	S	S	R	I	R	S	R
PS.28	R	R	R	R	S	S	R	R	R	R	R	R	R
PS.29	R	R	R	R	S	R	I	S	R	S	S	R	R
PS.30	R	R	S	S	R	R	S	R	R	R	R	S	R
PS.31	R	I	S	S	R	R	S	I	R	R	R	S	S
PS.32	R	I	S	S	R	R	S	S	R	R	R	R	R
PS.33	R	R	R	S	R	R	R	S	R	R	R	R	R
PS.34	S	R	S	S	S	S	S	S	I	I	S	I	R
PS.35	S	I	R	S	R	R	R	S	R	S	R	R	R
PS.36	S	S	S	R	R	R	S	I	I	R	R	S	R
PS.37	R	R	R	S	R	I	R	S	R	S	R	R	R
PS.38	R	S	S	R	R	R	S	S	S	I	R	S	R
PS.39	S	R	R	R	R	R	S	R	R	R	R	R	R
PS.40	R	R	R	R	S	R	R	S	R	S	R	R	R
PS.41	R	R	S	R	R	R	R	R	R	R	S	S	R
PS.42	S	S	S	R	R	R	R	I	R	S	R	S	R
PS.43	R	R	S	S	I	R	I	S	S	R	R	R	R
PS.44	R	R	R	R	R	S	S	S	R	R	R	R	I
PS.45	R	R	R	R	S	R	S	S	R	S	R	R	R
PS.46	R	I	R	R	R	R	S	R	R	S	R	R	I
PS.47	R	S	S	I	R	R	S	I	R	R	S	R	R
PS.48	R	R	R	S	R	I	R	S	S	R	R	R	R
PS.49	R	I	R	R	I	R	S	I	R	I	R	R	R
PS.50	R	R	R	I	R	R	S	R	R	R	R	R	R
PS.51	R	R	R	R	S	R	R	S	R	S	R	R	R
PS.52	R	R	R	S	R	R	S	R	R	R	R	R	R
PS.53	R	S	R	S	R	R	S	I	R	R	R	R	R
PS.54	R	R	S	S	R	R	I	I	R	R	R	R	R

R= Resistant S= Sensitive I= Intermediate