

Determination and Prevalence of Bacterial and Fungal Keratitis among Patients in Baghdad City

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The study included 234 patients attended to Ibn AL-Haithem hospital of ophthalmology in Baghdad city having microbial keratitis, at age of (18-81) years for the period from September 2017- April 2018. There were 147 (63%) patients with bacterial keratitis and 87 (37%) with fungal keratitis. Men with bacterial keratitis represented 60% (88) and women 40% (59), while men with fungal keratitis were 61% (53) and women 39% (34), the number of the patients with bacterial keratitis were 82(56%) and 51(59%) with fungal keratitis from Baghdad while the internally displaced were 62(44%) with bacterial and 36(41%) with fungal keratitis, the percentage of patients with bacterial keratitis and fungal keratitis were both (34%) at mean age of 73 ± 2.3 y. Most patients with bacterial keratitis recorded having diabetes mellitus(39%) while the diabetic with fungal keratitis represented (41%), The results revealed that gram positive bacteria was 102 (69%) and gram negative 45 (31%), *S.aureus* was the most common cause of bacterial keratitis 42(29%), followed by *S.epidermidis* 36(24%) then *Streptococcus spp.* 24(16%), *Pseudomonas spp.* 17 (12%), *Proteus, spp.* 13 (9%), *Escherichia coli* 9(6%) and *Enterobacter spp* 6(4%) while *Aspergillus fumigates* represented 26(30%) as the most common cause of fungal keratitis, *Aspergillus flavus* 19(22%), *Aspergillus niger* 16(18%), *Pencillium spp* 11(13%), *Fusarium oxysporium.* 8(9%), *Fusarium solani* 5(6%) and *Candida spp* 2(2%). *S. aureus* isolates were susceptible to Cloramphenicol and Ciprofloxacin (83%) while they were resistant to Clindamycin (57%). *S.epidermidis* isolates were susceptible to Cloramphenicol and Amikacin (86%), *Streptococcus spp* were susceptible to Tetracycline and Clindamycin (88%, 83%), *Pseudomonas spp* showed their high susceptibility to Tetracycline(96%) followed by Cloramphenicol and Ciprofloxacin (88%) both each, *Enterobacter spp* were susceptible to Tetracycline and Gentamicin (100%) while *E.coli* were susceptible to Cloramphenicol (100%) followed by Ciprofloxacin and Gentamicin (89%) both, fungal isolates were susceptible to all types of antibiotics used in the study as (98%) of *Aspergillus spp* isolates were sensitive to Itraconazole (98%) Voriconazole, Amphotericin B and Natamycin (95%,93%, 92%) respectively, *Pencillium spp.* were sensitive to Amphotericin B (100%) then Natamycin and Itraconazole (91%) both, *Fusarium spp* were sensitive to Natamycin and Itraconazole (100%), *Candida* isolates were sensitive (100%) to Amphotericin B, Natamycin and Voriconazole.

Keywords: Bacterial and fungal keratitis, Antibiotic susceptibility, Ibn Al-Haitham hospital of ophthalmology Baghdad ,Iraq.

Microbial keratitis is defined as a corneal infection which caused by a range of non-viral pathogens, including bacteria, protists and fungi¹. It characterized by pain and corneal ulceration with tissue infiltration, the vision will be affected according to the size and location of the ulcer².

The climate and socio-economic factors play an important role in the prevalence of each causative organisms, fungal infection is the main cause of corneal blindness, associated with agricultural injury³. Saprophytic fungi reported as a causative agents and *Candida albicans* was considered pathogenic in 45.8% of keratitis⁴. Hosted bacteria might infect the cornea, while the others can be found as normal flora on the lid margin⁵.

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The more virulent bacteria cause corneal destruction and it may be complete in 24-48 hours which will lead to ulceration, abscess, surrounding corneal edema and anterior segment inflammation⁶.

Ocular surface disease such as trauma, surgery, topical steroid also increased wearing of contact lens and refractive surgery are additional risk factors⁷. In Iraq, the common risky factors for microbial keratitis are corneal abrasions, and ocular surface disorders such as dry eye, trichiasis, old scars and most of them are sequels of cicatricial trachoma⁸.

The study s aimed to determine the prevalence of microbial keratitis infection causatives and determination the susceptibility of the isolates to the most common antibiotics used .

Patients and Methods

This study was conducted in Ibn Al-Haitham hospital of ophthalmology in Baghdad, A total of 234 patients attended the hospital from September 2017- Apri 2018, the patient's agreement was taken and information documented directly including the socio-demographic characters, age, and history of any disease.

The slit Lamp method was used to record the size and depth of the ulcer and the stromal suppuration. Height of hypopyon if present was also recorded⁹.

The scraping was divided into two ports bacterial and fungal detection, blood agar (Himedia, India), chocolate agar, brain heart-infusion agar (Salucea/Germany), MacConkey agar (BDH/England), Sabaroud agar (Biolife / Italy) and Gram reaction. After incubation, positive microbial cultures were further biochemical reaction were done to identified the species as most *Staphylococcus* spp. and *Pseudomonas* spp. were identified by API system (bio Merieux)¹⁰.

lacto phenol-cotton blue stain was used for examination of hyphae and spore morphological identification under the microscope¹¹.

Antibiotic susceptibility

Antibiotic susceptibility was monitored with the disk diffusion assay (Kirby-Bauer), the zone of inhibition was interpreted according to NCCLS guidelines¹².

The following antibiotics were tested Erythromycin (15 µg), Clarithromycin (15 µg), Chloramphenicol (30 µg), Clindamycin (2 µg), Tetracycline, (30 µg), Doxycycline

(30 µg), Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ceftriaxone (30 µg) supplied by HiMedia Lab.(India) , reference strains of “*Staphylococcus aureus* ATCC25923, *E. coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853” were used as a control . Antifungal drugs susceptibility was tested by using CLSI M38-A2 BMD method¹³. The fungal isolates were tested for susceptibility to four antifungal agents: amphotericin B, natamycin, Itraconazole and voriconazole, “ *Candida krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019” were used as a controls .

Statistical Analysis

Chi-square test for statistical analysis. A P value <0.05 was used for finding the significance difference between the groups and correlated t-test was used to find the relation between the variables within the same group by using spss program¹⁴.

RESULTS

Out of 234 patients having microbial keratitis , there were 147 (63%) patients with bacterial keratitis and 87 (37%) with fungal keratitis as shown in figure 1. Men with bacterial keratitis represented 60% (88) and women 40% (59) ,while men with fungal keratitis were 61% (53) and women 39% (34) as shown in figure 2. The results reveled that the number of the patients with bacterial keratitis were 82(56%) and 51(59%) with fungal keratitis from Baghdad while the internally displaced were 62(44%) and 36(41%) with bacterial and fungal keratitis, there was a significant differences between the two group as shown in table 1.

The results showed that the highest percentage of patients with bacterial keratitis and fungal keratitis were both (34%) at mean age of 73 ± 2.3 y while the lowest percentage of patients with bacterial keratitis was (18%) and with fungal keratitis (15%) at mean age of 25.5 ± 1.4 y as showed in table 2. Most patients with bacterial keratitis recorded having diabetes mellitus(39%) and the rest of them having arthritis and hypertension (31% ,30%) while the diabetic with fungal keratitis represented (41%) followed by arthritis and hypertension (35%,24%) as showed in table 3.

The results revealed that gram positive bacteria was 102 (69%) while gram negative 45 (31%), *S.aureus* was the most common cause of bacterial keratitis 42(29%), followed by *S.epidermidis* 36(24%) then *Streptococcus spp.* 24(16%) , *Pseudomonas spp.* 17 (12%) , *Proteus, spp.* 13 (9%) , *Escherichia coli* 9(6%) and *Enterobacter spp* 6(4%) while *Aspergillus fumigates* represented 26(30%) as the most common cause of fungal keratitis , *Aspergillus flavus* 19(22%), *Aspergillus niger* 16(18%) , *Pencillium spp* 11(13%) , *Fusarium oxysporium.*

8(9%), *Fusarium solani* 5(6%) and *Candida spp* 2(2%) as shown table 4.

The results of antibiotic susceptibility showed that *S. aureus* isolates were susceptible to Cloramphenicol and Ciprofloxacin (83%) followed by Doxycycline (81%) and Amikacin (74%) then Gentamycin , Erythromycin and Tetracycline (69%,64% ,62%) , while they were resistant to Clindamycin (57%) .*S.epidermidis* isolates were susceptible to Cloramphenicol and Amikacin (86%) then Erythromycin and Doxycycline (83%) followed by Clindamycin ,

Table 1. Distribution of the patients with bacterial ocular infection according to the Socio-demographic characters

Socio-demographic characters	Patients with bacterial keratitis	Patients with fungal keratitis
Baghdad	82(56%)	51(59%)
IDP	62(44%)	36(41%)
Total	147(100%)	87(100%)

IDP internally displaced persons, p value (< 0.05)

Table 2. Disrebuton of bacterial and fungal keratitis according to age group

Age/years	Mean±SD	Number of patients with bacterial keratitis %	Number of patients with fungal keratitis %
18-33	25.5±0.4	26(18%)	13(15%)
34-49	41.5±0.6	32(22%)	20(23%)
50-65	57.5±0.1	38(26%)	24(28%)
66-81	73.5±0.3	51(34%)	30(34%)

p value (< .05)

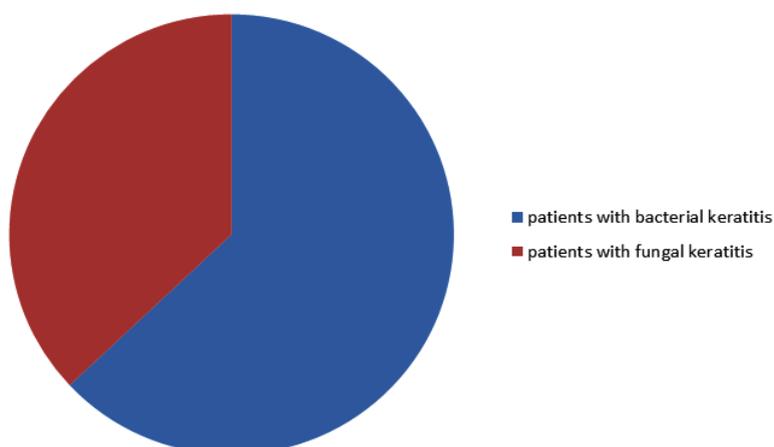


Fig. 1. Distribution of microbial keratitis among the patients

Ciprofloxacin and Gentamycin (81% ,78%,75%) respectively as shown in table 5 .

Streptococcus spp isolates were susceptible to Tetracycline and Clindamycin (88% ,83%) then Cloramphenicol (79%) followed by Erythromycin and Doxycycline (75%) both as shown in table 6.

Pseudomonas spp isolates showed their high susceptibility to Tetracycline(96%) followed by Cloramphenicol and Ciprofloxacin (88%) both and Amikacin ,Doxycycline and Gentamycin(82%) each of them as shown in table 7.

The results showed that *Enterobacter spp* isolates were susceptible to Tetracycline and Gentamicin (100%) followed by Ciprofloxacin (83%) while *E.coli* isolates were susceptible to Cloramphenicol (100%) followed by Ciprofloxacin and Gentamicin (89%) both and they were resistant to Amikacin and Tetracycline (56%) both as shown in table 8.

Proteus spp isolates were susceptible to Cloramphenicol and Tetracycline (92%) followed by Ciprofloxacin (85%) and Ceftriaxone and Gentamicin (77%) as shown in table 9.

Antifungal susceptibility test showed that (98%) of *Aspergillus spp* isolates were sensitive to Itraconazole (98%) , Voriconazole , Amphotericin B and Natamycin (95%,93% ,92%) respectively, *Pencillium spp.* isolates were sensitive to Amphotericin B (100%) then Natamycin and Itraconazole (91%) both followed by Voriconazole (82%) , as shown in table 10 .

Fusarium spp isolates were sensitive to Natamycin and Itraconazole (100%) then Amphotericin B and Voriconazole (92%) both as shown in table 11 .

The results revealed that *Candida* isolates were sensitive mostly to all types of the antibiotics were used in the study as showed in table 12.

Table 3. Relation between the number of patients with microbial keratitis and history of disease

History of disease	Number of patients with bacterial keratitis %	Number of Patients with fungal keratitis %
Diabetes mellitus	57(39%)	36(41%)
Arthritis	46(31%)	30(35%)
Hypertension	44(30%)	21(24%)
Total	147(100%)	87(100%)

p value (< 0.05)



Fig. 2. Distribution of bacterial and fungal keratitis between men and women

DISCUSSION

Our study revealed that patients with bacterial keratitis were represented (63%) from the total patients with microbial keratitis ,men represented the highest levels (60%) while fungal keratitis patients were (37%) and men represented (61%) of them The relatively highest percentage of males with microbial keratitis as a compression with females seen almost closely to some other studies^{15,16}. According to the socio-demographic characters , the number of patients with microbial keratitis from Baghdad represented (57%) while the internally displaced were (43%) and this might be related to unsuitable health conditions or inappropriate life issues .

There was high percentage of patients with microbial keratitis at mean age of 73.5±0.3 and gradually decreases and these fining are different in other study in Baghdad , as the patients group (41-59) years were the most microbial keratitis infected with a percentage of (30.20 %), while the lowest infected (e” 60) year with

a percentage of (17.80 %) and another study in south India they found that the age range of 41–60 years was more affected , this may be due to choosing of the common causes of infection¹⁷ .¹⁸ . The patients with bacterial keratitis recorded with diabetes mellitus were (39%) and the rest were having arthritis and hypertension (31% ,30%) while the diabetic with fungal keratitis were (41%) followed by arthritis and hypertension (35% ,24%) and these results disagree with other findings depending on the age and other chronic disease due to health style (food and environment)^{19,20} . The results revealed that gram positive bacteria was 102 (69%) and gram negative 45 (31%) , *S.aureus* was the most common cause of bacterial keratitis42(29%), followed by *S.epidermidis* 36(24%) then *Streptococcus spp.* 24(16%) , *Pseudomonas spp.* 17 (12%) , *Proteus, spp.* 13 (9%) , *Escherichia coli* 9(6%) and *Enterobacter spp* 6(4%) while the most common causes of fungal keratitis were *Aspergillus fumigates* 26(30%), *Aspergillus flavus* 19(22%) , *Aspergillus niger* 16(18%) , *Pencillium spp* 11(13%) , *Fusarium*

Table 4. Profile of isolated bacteria and fungi from patients with keratitis

Types of isolated bacteria	Number(%)	Types of isolated fungi	Number(%)
<i>S.aureus</i>	42(29%)	<i>Aspergillus fumigatus</i>	26(30%)
<i>S.epidermidis</i>	36(24%)	<i>Aspergillus flavus</i>	19(22%)
<i>Streptococcus spp.</i>	24(16%)	<i>Aspergillus niger</i>	16(18%)
<i>Pseudomonas spp.</i>	17(12%)	<i>Pencillium spp.</i>	11(13%)
<i>Proteus spp.</i>	13(9%)	<i>Fusarium oxysporium</i>	8(9%)
<i>Escherichia coli</i>	9(6%)	<i>Fusarium solani</i>	5(6%)
<i>Enterobacter spp</i>	6(4%)	<i>Candida spp</i>	2(2%)
Total	147(100%)	Total	87(100%)

Table 5. Antibiotic sensitivity and resistance of *S. aureus* and *S.epidermidis* isolates .

Antibiotics	<i>S. aureus</i> n=42					<i>S.epidermidis</i> n=36				
	MIC (µg/ml)		S	I	R	MIC (µg/ml)		S	I	R FRR
	50	90				50	90			
Erythromycin	0.25	64	27(64%)	2(5%)	13(31%)	0.25	64	30(83%)	1(3%)	5(14%)
Cloramphenicol	0.25	64	35(83%)	2(5%)	5(12%)	0.12	64	31(86%)	1(3%)	4(11%)
Clindamycin	0.06	64	16(38%)	2(5%)	24(57%)	0.06	32	29(81%)	2(6%)	5(14%)
Tetracycline	1	4	26(62%)	1(2%)	15(36%)	1	4	17(47%)	3(8%)	16(44%)
Amikacin	0.12	32	31(74%)	1(2%)	10(24%)	0.25	16	31(86%)	2(6%)	3(8%)
Gentamycin	0,25	8	29(69%)	2(5%)	11(26%)	0.25	16	27(75%)	3(8%)	7(19%)
Ciprofloxacin	8	8	35(83%)	2(5%)	5(12%)	8	8	28(78%)	2(6%)	6(17%)
Doxycycline	0.12	16	34(81%)	2(5%)	6(14%)	0.12	16	30(83%)	2(6%)	4(11%)

oxysporium. 8(9%) , *Fusarium solani* 5(6%) and *Candida spp* 2(2%) , these were agreed with other study in Baghdad as gram-positive isolates were (81.1%) from the total cases and *Staphylococcus spp.* was the most common isolates, *Pseudomonas spp.* was the first among gram-negative isolates²¹. Other study conducted in Ibn Al-Hiatham Teaching Eye Hospital at 2003, found that *Pseudomonas spp.* was the most common bacterial isolates, followed by *Staphylococcus spp.* and fungal growth was detected in 13 cases²² Other study founded

coagulase negative staphylococci were the most common may be related to the variation to different climatic conditions, socioeconomic variables²³. Another study found that the fungal growth represented (18.7%), and *Aspergillus spp.* was 42 (56.8%) followed by *Fusarium spp.* 20 (27%), *Penicillium spp.* 4 (5.4%), *Scopulariopsis spp.* 2 (2.7%), *Geotrichum spp.* 1 (1.4%), *Alternaria spp.* 1 (1.4%) and *Candida spp.* 4 (5.4%)²⁴. other investigation found that the prevalence of fungal keratitis in Iraq the most common fungus isolated

Table 6. Antibiotic sensitivity and resistance of *Streptococcus spp* isolates

Antibiotics	MIC (µg/ml)		<i>Streptococcus spp.</i> n=24		
	50	90	S	I	R
	Erythromycin	0.12	64	18(75%)	2(8%)
Clarithromycin	0.25	64	17(70%)	2(8%)	5(21%)
Cloramphenicol	0.25	64	19(79%)	1(4%)	4(17%)
Clindamycin	0.12	64	20(83%)	1(4%)	3(13%)
Tetracycline	0.12	64	21(88%)	2(8%)	1(4%)
Doxycycline	0.25	64	18(75%)	2(8%)	4(17%)
Ciprofloxacin	1	2	15(63%)	3(13%)	6(25%)
Ceftriaxone	0.5	4	16(65%)	1(4%)	7(29%)

Table 7. Antibiotic sensitivity and resistance of *Pseudomonas spp* isolates

Antibiotics	MIC (µg/ml)		<i>Pseudomonas spp.</i> n=17		
	50	90	S	I	R
	Amikasin	8	32	14 (82%)	1(6%)
Cloramphenicol	1	2	15(88%)	1(6%)	1(6%)
Tetracycline	8	32	16 (94%)	0	1(6%)
Doxycycline	1	2	14(82%)	1(6%)	2(12%)
Ciprofloxacin	1	16	15(88%)	1(6%)	1(6%)
Gentamicin	2	8	14(82%)	2(12%)	1(6%)

Table 8. Antibiotic sensitivity and resistance of *Enterobacter spp* and *E.coli* isolates

Antibiotics	<i>Enterobacter spp</i> n=6					<i>E.coli</i> n= 9				
	MIC (µg/ml)		S	I	R	MIC (µg/ml)		S	I	R FRR
	50	90				50	90			
Amikacin	8	32	3(50%)	1(17%)	2(33%)	16	32	4(44%)	0	5(56%)
Cloramphenicol	1	2	3(50%)	1(17%)	2(33%)	1	2	9(100%)	0	0
Tetracycline	8	32	6(100%)	0	0	8	8	4(44%)	0	5(56%)
Doxycycline	1	2	3(50%)	1(17%)	2(33%)	1	2	7(78%)	0	2(22%)
Ciprofloxacin	1	16	5(83%)	1(17%)	0	64	64	8(89%)	1(11%)	0
Gentamicin	2	8	6(100%)	0	0	2	8	8(89%)	1(11%)	0

was *Aspergillus* spp. then *Fusarium* spp., *Candida albicans* and *Scopulariopsis* spp²⁵. Our results revealed that gram positive and negative bacterial isolates were susceptible to the most antibiotics used as *S. aureus* isolates were susceptible to Cloramphenicol and Ciprofloxacin (83%) followed by Doxycycline (81%), while they were

resistant to Clindamycin (57%). *S. epidermidis* isolates were susceptible to Cloramphenicol and Amikacin (86%). *Streptococcus* spp isolates were susceptible to Tetracycline and Clindamycin (88%, 83%), *Pseudomonas* spp isolates showed their high susceptibility to Tetracycline(96%) followed by Cloramphenicol and Ciprofloxacin (88%) and

Table 9. Antibiotic sensitivity and resistance of *Proteus* spp isolates

Antibiotics	<i>Proteus</i> spp n=13				
	MIC (µg/ml)		S	I	R
	50	90			
Ceftriaxone	0.12	64	10 (77%)	2(15%)	1(8%)
Cloramphenicol	0.25	64	12(92%)	1(8%)	0
Tetracycline	8	8	12(92%)	1(8%)	0
Ciprofloxacin	16	16	11(85%)	1(8%)	1(8%)
Gentamicin	0.12	64	10(77%)	2(15%)	1(8%)

Table 10. Antibiotic sensitivity and resistance of *Aspergillus* spp and *Pencillium* spp isolates

Antifungal	<i>Aspergillus</i> spp. n= 61					<i>Pencillium</i> spp. n= 11		
	MIC (µg/ml)		S	I	R	S	I	R
	50	90						
Amphotericin B	2	4	57(93%)	1(2%)	3(5%)	11(100%)	0	0
Natamycin	32	32	56(92%)	2(3%)	3(5%)	10(91%)	1(9%)	0
Itraconazole	0.125	0.25	60(98%)	1(2%)	0	10(91%)	1(9%)	0
Voriconazole	0.25	0.5	58(95%)	1(2%)	2(3%)	9(82%)	2(18%)	0

Table 11. Antibiotic sensitivity and resistance of *Fusarium* spp

Antifungal	<i>Fusarium</i> spp. n= 13				
	MIC (µg/ml)		S	I	R
	50	90			
Amphotericin B	2	4	12(92%)	1(8%)	0
Natamycin	32	32	13(100%)	0	0
Itraconazole	0.125	0.25	13(100%)	0	0
Voriconazole	0.25	0.5	12(92%)	1(8%)	0

Table 12. Antibiotic sensitivity and resistance of *Candida* spp isolates

Antifungal	<i>Candida</i> spp. n= 2				
	MIC (µg/ml)		S	I	R
	50	90			
Amphotericin B	2	4	2(100%)	0	0
Natamycin	32	32	2(100%)	0	0
Itraconazole	0.125	0.25	1(50%)	1(50%)	0
Voriconazole	0.25	0.5	2(100%)	0	0

these were agreed with other studies in China and Iran^{26,27}. *Enterobacter spp* isolates were susceptible to Tetracycline and Gentamicin (100%) while *E.coli* isolates were susceptible to Cloramphenicol (100%) but they were resistant to Amikacin and Tetracycline (56%) both, however, *Proteus spp* isolates were susceptible to Cloramphenicol and Tetracycline (92%) and these findings were recorded in many other studies^{28,29}. fungal isolates showed high susceptibility as *Aspergillus spp* isolates were sensitive to Itraconazole (98%) , Voriconazole then Amphotericin B and Natamycin (95%,93% ,92%) respectively , *Pencillium spp.* isolates were sensitive to Amphotericin B (100%) then Natamycin and Itraconazole (91%) both while *Fusarium spp* isolates were sensitive to Natamycin and Itraconazole (100%) then Amphotericin B and Voriconazole (92%) both and *Candida* isolates were sensitive to the most antibiotics that used in the study ,these results were matching to many other results in the same field^{30,31}.

CONCLUSIONS

Keratitis was very clear among the patients with ocular bacterial infections. *S. aureus* was the highest gram positive bacterial isolates followed by *S.epidermids* while gram negative dominant isolates were *Pseudomonas spp* . *Aspergillus spp* recorded high prevalence followed by *Pencillium spp.* Gram positive and negative isolates as well fungal isolates were susceptible to the most common tested antibiotics which will support the treatment strategies and control the common practice of self medication.

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