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Acute Conjunctivitis: Antibiotic Susceptibility and Resistance to Different Antibiotics in Main Eye Hospital, Tripoli, Libya

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ABSTRACT
Acute conjunctivitis is characterized by inflammation of the conjunctiva of less than 3 to 4 weeks duration, cellular infiltration, and exudation. It may also result in corneal, lid, or orbital involvement which may lead to various complications. Bacterial conjunctivitis is a self-limiting process, but topical antibiotic treatment is recommended to eradicate the pathogen and reduce symptom duration. A hospital-based prospective study was conducted in the emergency and casualty department in Zawyat Aldahmani Eye Hospital, Tripoli /Libya comprising out-patients (OPD) visiting the department from June to December 2019 (186 cases). The primary objective was to evaluate the bacteriological pattern and the secondary objectives were to evaluate seasonal variation and antibiotic sensitivity pattern of the isolates. Conjunctival swabs included patients from both sexes (males n_106 and females n_80) and different ages; 80.1% of adults of the whole studied cases and the rest were children under 12 years old. Staph. hominis (26.17%) was the predominant organism isolated throughout the study. The commonest single organism isolates were Staph. haemolyticus (11.21%) Staph. aureus (9.35%). All isolates were identified and susceptibility patterns towards about 34 different antibiotics were studied. There were 186 patients enrolled. Of all cultures, 57.5% (107) yielded one of the study pathogens. Gram-positive organisms were isolated from 83 (77.57%) of cultures, gram-negative organisms were isolated from 15 (14.02%), and 9 (8.4%) were fungal growth. From 107 gram-positive isolates, 19 samples (22.9%) gave coagulase-positive results and 64 samples (77.1%) gave negative results. All the major organisms (S. aureus, S. hominis, S. hemolytic, S. epidermidis, E. coli, Mycobacterium, and Moraxilla catarrhalis) susceptible to cephalosporins, macrolides, aminoglycosides and fluoroquinolones (levofloxacin). Tetracycline has the lowest effect on both gram-positive and gram-negative isolates. In conclusion, our findings should alert physicians on the choice of appropriate antibiotic treatment after applying AST to the identified strains and on the potential role of conjunctivitis in the spread of antibiotic-resistant pathogens. In addition, external bacterial infections of the eye were most frequently caused by the common skin bacteria. Staphylococci spp. was by far the most common bacterial pathogen.

Keywords: Acute bacterial conjunctivitis, Antimicrobial sensitivity, seasonal variations.

INTRODUTION

Bacterial conjunctivitis is one of the most common forms of ocular diseases worldwide. Conjunctivitis is a common ocular surface disease that is often divided into two types; infectious and non-infectious [(1)], chronic and acute. Acute conjunctivitis is generally defined as conjunctivitis with symptoms persists less than 3–4 weeks [(2, 3)].

Recently the discovery of significant of antibiotic resistance amongst pathogens especially ocular pathogens is a concern [(4-7)]. Commonly failure of treatment in most cases attributed to antibiotic resistance that complicates the selection of suitable antibiotic for treatment [(8-10)]. The first step in starting an antibiotic therapy is the identification of causative pathogen and assessment of their antibiotic resistance profiles [(11)]. Although cultures are performed for vision-threatening ocular infections, they are occasionally performed for routine eye infections, with doctors preferring empirical therapy to avoid treatment delays associated with the time required to obtain culture and sensitivity results and/or to avoid the costs of culturing from economic point of view. In the absence of culture and sensitivity results, antibiotic resistance data from surveillance studies can inform the choice of initial or empirical treatment [(12)]. In 2005 Ocular TRUST in USA (The Ocular Tracking Resistance in US Today) a nationwide investigation program was established to monitor antibiotic resistance specific to common ocular pathogens from 2005-2008 [(13, 14)]. Results showed high levels of methicillin resistance among *staphylococci*, with a predominance of concurrent resistance to other antibiotic classes. The extension of this study in multicenter nationwide surveillance in USA among clinically relevant isolates of *Staphylococcus aureus*, coagulase-negative *staphylococci* (CoNS), *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Haemophilus influenza* data have provided clinicians with an understanding of antibiotic resistance patterns to aid in antibiotic selection and ultimately improve patient outcomes [(15-17)]. Furthermore, an improvement in rapid diagnostic tests such as the use of BD phoenix™ automated microbiology system provides several collaborative opportunities for diagnostic laboratory teams, especially where the delay in the beginning of appropriate therapy has significant consequences for patient outcomes. These instruments contribute in the production of accurate organism identification and timely antimicrobial susceptibility testing data [(18)]. Assessments that provide accurate organism identification and antimicrobial susceptibility not only benefit the individual patient but also increase the effectiveness of antimicrobial surveillance programs. Here comes the need of the study. The primary objective of our study was to evaluate the bacteriological pattern in culture positive cases of acute bacterial conjunctivitis. The secondary objectives were evaluation of seasonal variation of organism profile and antibiotic sensitivity profile of different organisms.

MATERIAL AND METHODS

Clinical isolates

A hospital based cross sectional study was conducted in the department of emergency and casualty in Zawyat Aldahmani eye hospital, Tripoli /Libya. A total of 186 isolates from conjunctival swabs of out-patients visiting the department in the period from June to December 2019 were collected (duration of study: 7 months). Analyzed variables include bacteriologic results of isolation and identification and susceptibility patterns towards about 34 different antibiotics using routine microbiology laboratory techniques and BD phoenix™ automated microbiology system.

Sampling of Ocular Surface Bacteria

Sample collection was performed in a clean ophthalmic consulting room. The eye discharge was collected by rolling sterile cotton swab (Bibby Sterilin Ltd., Stone, UK) over the conjunctiva of each eye respectively. Culture swab samples were taken from the posterior lid margin and lower conjunctival sac before being placed directly into a sterile swab holder containing Nutrient broth and taken to the laboratory of microbiology in the same hospital for microbiological investigation.

Microbial Culture

Each conjunctival culture sample was plated onto one chocolate agar, blood agar and MacConkey agar (Oxoid Ltd., Basingstoke, UK) within 2 hours of sampling. Blood agar and MacConkey agar plates were incubated at 35°C for 24 hours. For Chocolate agar it was incubated in 7% CO_2 atmosphere for the same time and temperature. Positive cultures were identified by gram staining, coagulase slide test.

Antibiotic susceptibility testing

By Kirby-Bauer disk-Diffusion susceptibility testing: Kirby-Bauer disc diffusion method was used for antibiotic susceptibility testing on Mueller Hinton agar according to CLSI recommendation. [18] Zone of inhibition was measured and the antibiotic sensitivity was reported as sensitive, intermediate or resistant to the specific antibiotic tested according to manufacturer guideline. This technique was performed for all isolates; five antibiotics were tested namely, Gentamicin, Tetracycline, Ciprofloxacin, Neomycin and Chloramphenicol.

Phoenix identification system: The Phoenix identification method uses modified conventional, fluorogenic, and chromogenic substrates. Research-use-only combination panels (NMIC/ID-26; catalog no. 448026) for both identification and susceptibility testing were used for this comparison. Software versions V3.34A and V3.54A were used for this study. The ID side contains 45 wells with dried biochemical substrates and 2 fluorescent control wells. The ID broth was inoculated with bacterial colonies from a pure culture adjusted to a 0.5 McFarland standard by using a CrystalSpec nephelometer (BD Diagnostics), according to the manufacturer's recommendations. A 25-µl aliquot of this suspension was removed for AST, and the remaining suspension was then poured into the ID side of the Phoenix panel. The specimen was logged and loaded into the instrument within the specified timeline of 30 min. Quality control was performed according to the manufacturer's recommendations.

Phoenix system antimicrobial susceptibility testing: The AST side of the combination panel contains up to 84 wells with dried antimicrobial panels and 1 growth control well. The assay is a broth-based microdilutions test. The system uses a redox indicator for the detection of organism growth in the presence of an antimicrobial agent. The previously described 25 µl of the standardized ID broth suspension was transferred to the AST broth, yielding a final concentration of approximately 5×10^5 CFU/ml. Quality control was performed according to the manufacturer's recommendations.

Ethical considerations

Ethical approval for the study was obtained from the institutional Ethics committee of Zawyat Al-dahmani Eye Hospital. Informed consent was taken from all participants and from legally acceptable representatives in case of children before enrolling into the study.

Statistical analysis

Continuous data was presented as mean ± SD or Median Range, whereas categorical data was represented as frequency.

RESULTS

The study is reported as per STROBE guidelines. [19] Participant flow chart is showed in figure 1.

We have screened 186 patients showing clinical signs and symptoms of acute bacteria conjunctivitis. Out of which, 107 patients came out to be culture positive and were included in the study.

Demographic characteristics

Total no of culture positive cases in our study were 107, 80.4% were adults. Single eye involvement were seen in 68.22% patients (n=73) and bilateral involvement was seen in 31.78% (n =34). Sex of included patients was 78 males and 29 females (data shown in table 1).

Figure 1Participant flow chart

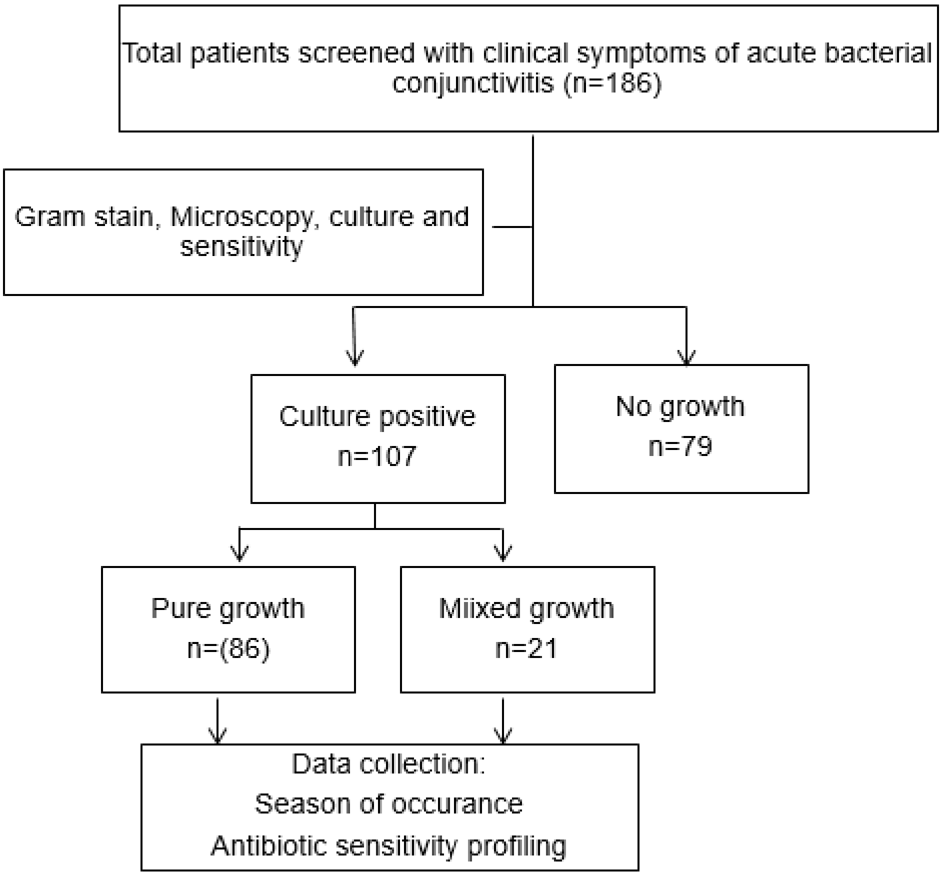


Table 1 Demographic characteristics of acute bacterial conjunctivitis

Total number of cases enrolled (n)		186
Age (years)	Adult	151 (80.1%)
	Child	35 (19.6%)
Sex	Male	106 (56.9%)
	Female	80 (43.1%)
Unilateral/Bilateral involvement	UL	73 (68.22%)
	BL	34 (31.78%)
Month of occurrence	June	34 (31.7%)
	July	29 (27.1%)
	August	21 (19.6%)

	September	11 (10.3%)
	October	5 (4.7%)
	November	5 (4.7%)
	December	2 (1.9%)

Seasonal variation

Seasons of occurrence of infection were different during the study period. Maximum number of cases was in June which consisted 31.8% of total cases. Increased frequencies were seen in the months of June (n=34, 31.8%), July (n=29, 27.1%) and August (n=21, 19.6%) however cases decreased in November (n=5, 4.7%) and December (n=2, 1.9%) in comparison with other months of the study.

Microbiological profile

Total number of culture positive cases in our study was 107 comprising 83 gram positive strains, 15 gram negative strains and 9 fungal isolates (fig. 2). Among gram positive strains were 22.9% coagulase positive isolates as shown in fig. 3. *Staph. aureus* (26.17%) was the predominant organism isolated throughout the period of study followed by *Staph. haemolyticus* (11.1%), *Staph. hominis* (9.35%), *Microbacterium* and fungal isolates (8.41% respectively), *E. coli* (7.48%), *Staph. epidermidis* (6.54%), *Moraxilla catarrhalis* (5.61%). Fungal isolates not identified in our study (Data shown in fig. 4).

Figure 2 Percentage of isolated microorganisms

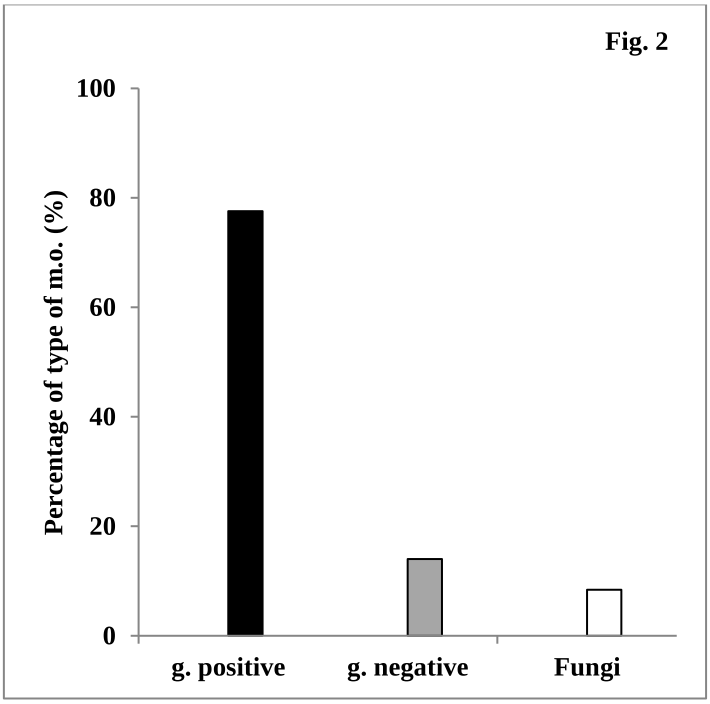
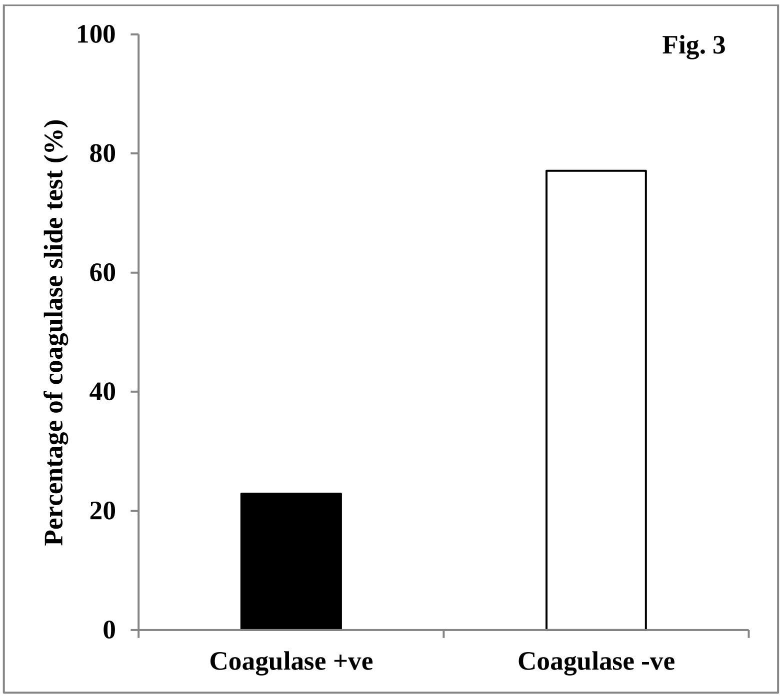


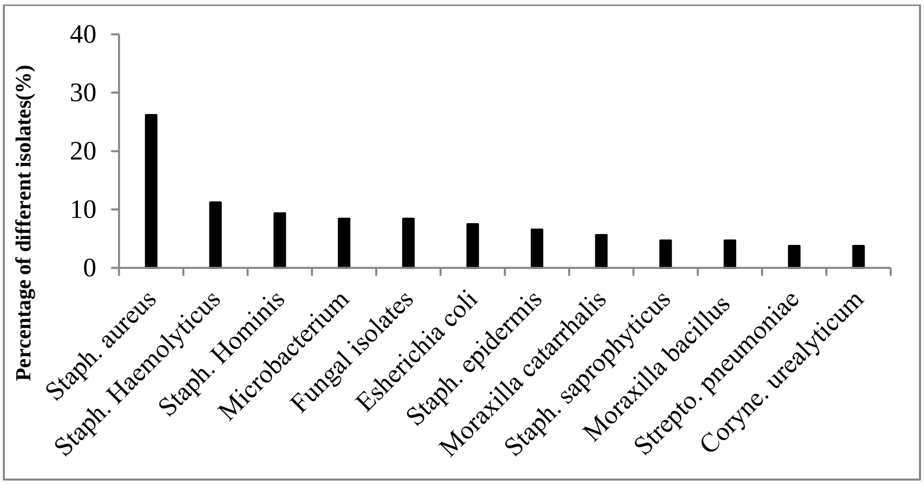
Figure 3 Percentage of coagulase positive isolates



Antibiotic sensitivity pattern

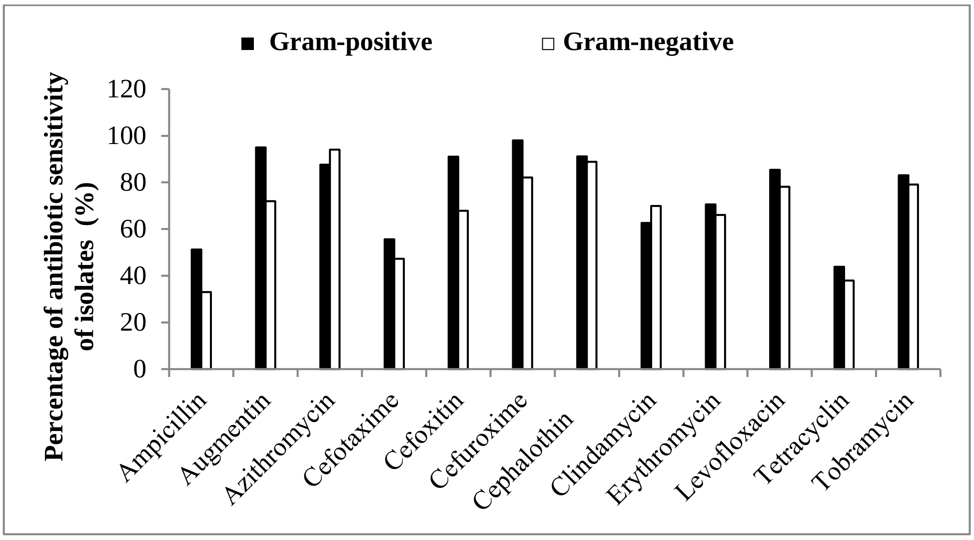
More than 90% of gram positive isolates were sensitive to cephalosporins (cefuroxime, cefoxitin and cephalothin) and augmentin. In addition high number of isolates (87.6% and 70.6%) were sensitive to macrolids (azithromycin and erythromycin) respectively.

Figure 4 Percentage of different isolated strains (98 bacterial isolates and 9 fungal isolates).



Additionally 85.4% of gram positive isolates were sensitive to flouroquinolone levofloxacin followed by tobramycin 83% of total isolates. However more than 40% of isolates was resistant to ampicillin, tetracycline, penicillin G and gentamicin. There was no significant difference in sensitivity pattern of gram negative isolates in this study for most tested antibiotics except towards some cephalosporins were the effects were less on gram negative strains. Conversely gram negative isolates were more sensitive towards azithromycin than gram positive isolates in this study (fig. 5).

Figure 5 antibiotic sensitivity patterns of various isolates (gram positive and gram negative)



Discussion

Conjunctivitis, inflammation of the mucosa of conjunctiva, is the most frequent ocular case with noticeable economic and social burdens (20). Bacteria contribute for about 50–70% of infectious conjunctivitis (21). In our study, out of 186 patients screened with signs and symptoms of acute bacterial conjunctivitis, 107 (57.5%) showed culture positivity. Median age of presentation was 29 year (Range 8–63 year). Bilateral eye involvement was seen in 31.78% cases. In our study maximum number of cases was seen in the month of June to August (78.4%). In the month of September to December, there was a decline trend of culture positive bacterial conjunctivitis cases were seen. Similar finding was reported by Aggarwal et al, where it was found that the frequency of bacterial conjunctivitis follows a step ladder pattern. During the summer its frequency was increased, sudden decreases in frequency was seen in autumn and remained low during winter season (22). Staphylococcus species were isolated through the whole study period, whereas *Moraxella bacillus* and *Corynebacterium urealyticum* were mainly isolated during September to December. In agreement with our findings and according to the studies conducted so far gram positive bacteria are associated with variety of ocular infections. The most commonly reported isolates belong to the genus Staphylococci regardless of the study area and population. Moreover Staphylococci are associated with any type of eye infections including conjunctivitis and keratitis (23, 24). Additionally, both *S. aureus* and CoNS (Coagulase-negative *Staphylococci*) took the highest proportion of the isolates (26.17%) in accordance with other studies reports (25, 26). Despite their normal existence, CoNS are the most frequent cause of ocular infections with increasing frequencies over time (27). A 5 year retrospective study in Iran indicated that 40% of infections were due to CoNS (28). A Similar study in India also found a prevalence of 45.4% (29). The problem is worse especially in preoperative and post-operative cases. In a study conducted in patients with cataract surgery, 88.8% of isolates from conjunctival swabs were CoNS (30). Likewise, 65.9% and 21% of pre-operative cataract patients had CoNS and *S. aureus* isolates respectively. Our findings are in accordance with previous literatures (25-30). Overall, *Staph. aureus* (26.17%) was the predominant organism followed by *Staph. Haemolyticus* (11.21%), *Staph. hominis* (9.35%), *Microbacterium* (8.41%), *E. coli* (7.48%), *Staph. epidermidis* (6.54%), *Moraxilla catarrhalis* (5.61%) in our study. *Staph.*

saprophyticus, *Moraxella bacillus* (4.67%) respectively and *Strepto. pneumoniae*, *Coryne. urealyticum* (3.74%) respectively were isolated less frequently in our study participants.

Studies from different parts of the world indicated that diverse groups of gram negative bacteria are isolated from ocular infections. Among gram negatives, frequent isolates of conjunctivitis include *P. aeruginosa*, *E. coli*, *Enterobacter* spp., *Proteus spp.*, *Moraxella spp.*, and *N. gonorrhoeae* (31, 32). In agreement with these findings in our study some of the same gram negative strains were isolated. Furthermore, Micrococcus, the gram positive organism attributed to 8.41% of isolates in our study was similarly contributed to 4.3% of ocular infections in a study conducted in Iran (33).

Most bacterial conjunctivitis are self-limiting (34), although topical antibiotics are recommended because they can shorten the duration of the disease (35) and prevent the spread of infection. Broad-spectrum antibiotics are generally used empirically as first-line therapy for bacterial conjunctivitis. In case of culture positive conjunctivitis cases, topical antibiotics seem to be more effective in achieving clinical and microbiological cure (36). In our study, most of the major isolates were sensitive

to cephalosporins, macrolids (azithromycin and erythromycin), flouroquinolone levofloxacin followed by tobramycin. However more than 40% of isolates were resistant to ampicillin, tetracycline, penicillin G and gentamicin.

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Authors' contributions

RM Conceptualization

RM and **HA** Data curation

RM and **NS** Collection of samples

RM, **SE** methodology

RM supervision and writing original draft

RM; **SE** and **HA** writing, review and editing

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