IRAQI Academic Scientific Journals
المجلات الاكاديمية العلمية العراقية
University of Kut Journal
ISSN (E): 2616 - 7808 II ISSN (P): 2414 - 7419
js.uokut.edu.iq
k.u.c.j.sci@alkutcollege.edu.iq
Vol. 11 , Issue 1 , June 2026

Comparative in vitro Activity of Selected Antibiotics and Probiotics against *Staphylococcus aureus* and *Streptococcus pyogenes* from Impetigo Cases in Wasit, Iraq

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Abstract

Impetigo is a common and contagious superficial skin infection, primarily caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. This study aimed to assess the in vitro activity of selected antibiotics and probiotics against *S. aureus* and *S. pyogenes* isolated from impetigo cases in Wasit, Iraq, and to compare their antimicrobial effects. In a cross-sectional study conducted from August to November 2024, 102 skin swabs were collected from patients with impetigo. Bacterial isolates were identified using standard microbiological methods and the Vitek® 2 system. Antibiotic susceptibility was determined by the disk diffusion method for 31 *S. aureus* and 6 *S. pyogenes* isolates against 17 antibiotics. The inhibitory activity of *Lactobacillus rhamnosus* and *Lactobacillus fermentum* was evaluated using the well diffusion and agar spot methods.*S. aureus* was the predominant pathogen (48.0%), followed by *S. pyogenes* (5.9%). *S. aureus* showed high resistance to penicillin G (90.3%) but remained susceptible to levofloxacin (100%) and vancomycin (96.8%). *S. pyogenes* exhibited 100% susceptibility to penicillin G but high resistance to tetracycline (66.7%). Both probiotic strains demonstrated significant antibacterial activity. *L. fermentum* showed larger inhibition zones (18 mm against *S. aureus*, 16 mm against *S. pyogenes* in the well diffusion method) compared to *L. rhamnosus*. The agar spot method proved more effective, with inhibition zones reaching up to 26 mm for *L. fermentum*. The study reveals significant antibiotic resistance among impetigo pathogens in Wasit, Iraq, highlighting the need for updated treatment guidelines. The potent in vitro antimicrobial activity of *L. rhamnosus* and *L. fermentum* suggests that probiotics could be a promising alternative or adjunctive therapy for impetigo, warranting further clinical investigation.

Keywords: Impetigo, *Staphylococcus aureus*, *Streptococcus pyogenes*, Drug Resistance, *Lactobacillus*

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Paper Info.
Published: Jun. 2026

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معلومات البحث
تأريخ النشر : حزيران 2026

دراسة مقارنة للفعالية المخبرية لمضادات حيوية وبروبيوتيك مختارة ضد بكتيريا *Staphylococcus aureus* و *Streptococcus pyogenes* المعزولة من حالات الحصف في واسط، العراق

احمد قنديل حاجم [1] ، جاسم حسين مخرمش [2]

المستخلص

الحصف هو عدوى جلدية سطحية شائعة ومعدية، تسببها بشكل أساسي بكتيريا *Staphylococcus aureus* و *Streptococcus pyogenes*. هدفت هذه الدراسة إلى تقييم الفعالية المخبرية لمضادات حيوية وبروبيوتيك مختارة ضد بكتيريا *S. aureus* و *S. pyogenes* المعزولة من حالات الحصف في واسط، العراق، ومقارنة تأثيراتها المضادة للميكروبات. في دراسة مقطعية أجريت من أغسطس إلى نوفمبر 2024، تم جمع 102 مسحة جلدية من مرضى الحصف. تم تحديد العزلات البكتيرية باستخدام الطرق الميكروبيولوجية القياسية ونظام Vitek® 2. تم تحديد حساسية المضادات الحيوية بطريقة انتشار القرص لـ 31 عزلة من *S. aureus* و 6 عزلات من *S. pyogenes* ضد 17 مضادًا حيويًا. تم تقييم النشاط المثبط لسلالتي *Lactobacillus rhamnosus* و *Lactobacillus fermentum* باستخدام طريقتي انتشار البئر وبقعة الأجار. كانت *S. aureus* هي الممرض السائد (48.0%)، تليها *S. pyogenes* (5.9%). أظهرت

S. aureus مقاومة عالية للبنسلين (90.3%) Gولكنها ظلت حساسة لليفوفلوكساسين (100%) والفانكومايسين (96.8%). أظهرت *S. pyogenes* حساسية بنسبة 100% للبنسلين G ولكن مقاومة عالية للتتراسيكلين (66.7%). أظهرت كلتا سلالتي البروبيوتيك نشاطًا كبيرًا مضادًا للبكتيريا. أظهرت *L. fermentum* مناطق تثبيط أكبر (18 ملم ضد *S. aureus*، و 16 ملم ضد *S. pyogenes* بطريقة انتشار البئر) مقارنة بـ *L. rhamnosus.* أثبتت طريقة بقعة الأجار أنها أكثر فعالية، حيث وصلت مناطق التثبيط إلى 26 ملم لـ *L. fermentum.* تكشف الدراسة عن مقاومة كبيرة للمضادات الحيوية بين مسببات الحصف في واسط، العراق، مما يؤكد الحاجة إلى تحديث الإرشادات العلاجية. يشير النشاط القوي المضاد للميكروبات في المختبر لسلالتي *L. rhamnosus* و *L. fermentum* إلى أن البروبيوتيك يمكن أن يكون علاجًا بديلاً أو مساعدًا واعدًا للحصف، مما يستدعي مزيدًا من البحث السريري.

الكلمات المفتاحية: الحصف، المكورات العنقودية الذهبية، المكورات العقدية المقيحة، مقاومة الأدوية، اللاكتوباسيلس

Introduction

Impetigo is a highly contagious superficial skin infection commonly seen in children, especially in hot humid areas. The most common type of bacteria responsible for the infection is *Staphylococcus aureus*, though *Streptococcus pyogenes* also plays a role **[1]**. Though impetigo is usually a self-limiting condition, antibiotic therapy is required to shorten the tenure of illness, halt the spread lesions and decrease complications like post-streptococcal glomerulonephritis [2].

The rise in antibiotic resistance rates among cutaneous pathogens is a major obstacle to successful impetigo management. Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an important issue, and community-acquired infections are increasing. Very high level of resistance has been reported to frequently used antibiotics like penicillin, erythromycin and clindamycin in *S. aureus* and *S. pyogenes* isolated from skin and soft tissue infections. This increasing resistance calls for the further study of other therapeutic approaches [3,4,5].

Probiotics, which are live microorganisms that provide a health benefit to the host, have been put forward as alternative and/or adjunctive means in treatment for skin infections. Topical Probiotics have been reported to modulate the skin microbiome, improve barrier function of the skin and suppress growth of pathogenic bacteria in multiple ways such as production of antimicrobials and competitive exclusion [6]. Some research has demonstrated the potential beneficial role of probiotics, especially *Lactobacillus* species, on prophylaxis and management of skin diseases related to *S. aureus* colonization [7].

Probiotics have also been shown to be a potentially effective therapy in the management of impetigo; however, there is paucity of clinical trials comparing it with standard antibiotics. In addition, there is a lack of local information on the antimicrobial susceptibility of *S. aureus* and *S. pyogenes* isolates causing impetigo in Iraq [7]. Thus, the present study aimed to fill in this gap and compare the in-vitro activity of some antibiotics and probiotics against clinical isolates of *S. aureus* and *S. pyogenes* isolated from patients with impetigo in Wasit Province, Iraq. The results of this study will contribute to the knowledge about localized resistance patterns and probiotic therapy as a treatment modality for impetigo, which are clinically significant gaps and research priorities in patient care.

Material and method

Study Design and Ethical Considerations

A cross-sectional study was conducted between

August and November 2024 in Wasit Province, Iraq. The study protocol was approved by the Institutional Review Board of the College of Medicine, Wasit University. Written informed consent was obtained from all adult participants and from the legal guardians of participating children. All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments.

Sample Collection

A total of 102 skin swab samples were collected from patients with a clinical diagnosis of impetigo. Sampling was conducted at Al-Zahra Teaching Hospital, Al-Karama Teaching Hospital, and several private dermatology clinics in Wasit. Participants ranged in age from 1 to 50 years. Specimens were collected from lesions on exposed body areas, such as the face, hands, and feet, using sterile swabs under aseptic conditions. The swabs were then placed in a sterile transport medium and immediately transported to the laboratory for processing.

Bacterial Isolation and Identification

Upon arrival at the laboratory, samples were cultured on Mannitol Salt Agar (MSA) and Blood Agar plates. The plates were incubated aerobically at 37°C for 24–48 hours. Preliminary identification of bacterial isolates was based on colony morphology, Gram staining, and a series of biochemical tests, including catalase, oxidase, coagulase (slide and tube methods), and bacitracin sensitivity. Final identification of the isolates was confirmed using the Vitek® 2 automated system (bioMérieux, France). Identified *S. aureus* and *S. pyogenes* isolates were preserved in Brain Heart Infusion (BHI) broth supplemented with 20% glycerol and stored at –20°C for further analysis.

Antibiotic Susceptibility Testing

The antibiotic susceptibility of the bacterial isolates was determined using the Kirby-Bauer disk diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024). A bacterial suspension equivalent to a 0.5 McFarland standard was prepared and uniformly streaked onto Mueller-Hinton agar plates. Antibiotic disks were placed on the agar surface, and the plates were incubated at 37°C for 18–24 hours. The diameters of the inhibition zones were measured in millimeters. The following antibiotics were tested: levofloxacin, tetracycline, trimethoprim, vancomycin, azithromycin, erythromycin, gentamicin, penicillin G, doxycycline, chloramphenicol, methicillin, linezolid, ofloxacin, clindamycin, clarithromycin, and ceftriaxone.

Probiotic Preparation

Two commercial probiotic strains, *Lactobacillus rhamnosus* (Vitamatic, USA) and *Lactobacillus fermentum* (Maple Life Sciences, India), were used in this study. The contents of each probiotic capsule were suspended in 10 mL of De Man, Rogosa, and Sharpe (MRS) broth and cultured on MRS agar. The plates were incubated anaerobically at 37°C for 48 hours to obtain pure cultures.

Assessment of Probiotic Activity

Well Diffusion Method

The antibacterial activity of the probiotics was evaluated using the well diffusion method. Both broth culture bacteria (BCB) and cell-free supernatant (CFS) of the *Lactobacillus* species were tested. The CFS was obtained by centrifuging the broth culture at 10,000 rpm for 15 minutes, followed by filtration of the supernatant through a 0.22 µm pore size filter [8]. Pathogenic bacterial suspensions (0.5 McFarland standard) were swabbed onto nutrient agar plates. Wells (6 mm in diameter) were created in the agar, and 50 µL of BCB or CFS from each probiotic strain were added to the wells. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones (ZDIs) was measured [9].

Agar Spot Method

An overnight culture of each *Lactobacillus* species in MRS broth was spotted onto the surface of MRS agar plates and incubated for 24 hours at 37°C. A semi-solid nutrient medium containing the pathogenic bacteria was then poured over the MRS agar. The plates were incubated for another 24 hours at 37°C, and the zones of inhibition were examined and measured [10]

Data Analysis

Data were analyzed using SPSS software (Version 25.0). Descriptive statistics were used to summarize the demographic and microbiological data. The chi-square test was used to assess associations between categorical variables. A p-value of ≤ 0.05 was considered statistically significant.

Result

A total of 102 patients with clinically diagnosed impetigo were included in this study. The patient population ranged in age from 1 to 50 years, with the highest incidence observed in the 1–10 age group (63.7%), followed by progressively lower rates in older age groups. The lowest incidence was recorded in the 41–50 age group (0.98%). A statistically significant association ($P \leq 0.05$) was found between patient age and the clinical presentation of impetigo. Non-bullous impetigo was the most prevalent clinical form, accounting for 62.7% of cases, followed by bullous impetigo (26.5%) and ecthyma (10.8%). As shown in Table (1).

The anatomical distribution of lesions revealed that exposed areas of the body were predominantly affected. The face was the most common site of infection (47.1%), followed by the foot (22.5%), hands (15.7%), gluteus (4.9%), trunk (3.9%), head (3.9%), and femoral region (2.0%). As shown in Table (2).

Table (1): Distribution of Impetigo Clinical Types Among Study Participants

Type of Infection	Frequency (n)	Percentage (%)
Non-bullous Impetigo	64	62.7
Bullous Impetigo	27	26.5
Ecthyma	11	10.8
Total	102	100.0

Table 2: **Anatomical Distribution of Impetigo Lesions Among Study Participants**

Specimen Area	Frequency (n)	Percentage (%)

Face	48	47.1
Foot	23	22.5
Hands	16	15.7
Gluteus	5	4.9
Trunk	4	3.9
Head	4	3.9
Femoral	2	2.0
Total	102	100.0

Among the 102 skin swab samples collected, *Staphylococcus aureus* was the most frequently isolated pathogen, identified in 49 (48.0%) of the cases. *Streptococcus pyogenes* was detected in 6 (5.9%) isolates, confirming their roles as the primary etiological agents of impetigo in this population. Other Gram-positive bacteria were isolated in 31.4% of cases, while no bacterial growth was observed in 14.7% of the samples.

The antibiotic susceptibility testing of 31 *S. aureus* isolates revealed the following results: all isolates (100%) were sensitive to levofloxacin and chloramphenicol. High sensitivity was also observed for vancomycin (96.8%), doxycycline (87.1%), gentamicin (83.9%), and methicillin (83.8%). Moderate sensitivity was noted for trimethoprim (80.5%), azithromycin (67.8%), and tetracycline (61.3%). However, high resistance rates were observed for penicillin G (90.3%), erythromycin (35.4%), and azithromycin (29.0%). The results were statistically significant ($P \leq 0.001$). As shown in Table (3).

Table (3): Distribution of *S. aureus* based on antibacterial sensitivity

Antibiotics	Results			Total
	Sensitive No.(%)	Intermediate No.(%)	Resistant No.(%)	
Levofloxacin (LEV)	31(100)	0(0)	0(0)	31
Tetracycline (TE)	19(61.3)	5(16.1)	7(22.6)	31
Trimethoprime(TM)	25(80.5)	2(6.5)	4(13)	31
Vancomycin (VA)	30(96.8)	0(0)	1(3.2)	31
Azithromycin (AZM)	21(67.8)	1(3.2)	9(29)	31
Erythromycin(E)	16(51.6)	4(13)	11(35.4)	31
Gentamycin (GEN)	26(83.9)	0(0)	5(16.1)	31
Penicillin G (P)	3(9.7)	0(0)	28(90.3)	31
Doxycycline (DXT)	27(87.1)	3(9.7)	1(3.2)	31
Chloramphenicol (C)	31(100)	0(0)	0(0)	31

Methicillin (MET)	26(83.8)	5(16.2)	0(0)	31

P≤0.001, Pearson Chi-Square =167.157

All 6 *S. pyogenes* isolates (100%) were sensitive to linezolid, ofloxacin, chloramphenicol, clindamycin, erythromycin, azithromycin, clarithromycin, levofloxacin, vancomycin, and penicillin G. However, resistance was observed for tetracycline (66.7%) and ceftriaxone (33.3%). The results were statistically significant ($P \leq 0.001$). As shown in Table 4

Table (4): Distribution of *S. pyogenes* based on antibacterial sensitivity

Antibiotics	Sensitive No.(%)	ResistantNo.(%)	Total
Linezolid (LNZ)	6(100)	0(0)	6
Ofloxacin (OFX)	6(100)	0(0)	6
Chloramphenicol (C)	6(100)	0(0)	6
Clindamycin (DA)	6(100)	0(0)	6
Erythromycin(E)	6(100)	0(0)	6
Azithromycin (AZM)	6(100)	0(0)	6
Clarithomycin (CLR)	6(100)	0 (0)	6
Tetracycline (TE)	2(33.3)	4(66.7)	6
Levofloxacin (LEV)	6(100)	0(0)	6
Vancomycin (VA)	6(100)	0(0)	6
Penicillin G (P)	6(100)	0(0)	6
Ceftriaxone (CRO)	4(66.7)	2(33.3)	6

P≤0.001, Pearson Chi-Square = 37.091

The antimicrobial activity of *Lactobacillus* species was evaluated using both broth culture bacteria (BCB) and cell-free supernatant (CFS). *L. fermentum* demonstrated inhibition zones of 18 mm (BCB) and 13 mm (CFS) against *S. aureus*, and 16 mm (BCB) and 12 mm (CFS) against *S. pyogenes*. *L. rhamnosus* produced inhibition zones of 14 mm (BCB) and 12 mm (CFS) against *S. aureus*, and 15 mm (BCB) and 12 mm (CFS) against *S. pyogenes*. The results were statistically significant ($P \leq 0.05$). As shown in Tables 5and 6

Table (5): Antimicrobial effect of *Lactobacilli* spp. in millimeters using well diffusion method by bacterial culture broth (BCB)

Isolates	Probiotic		Total No.(%)
	L. rhamnosus DZI by BCB (mm) No.(%)	*L. fermentum* DZI by BCB (mm) No.(%)	
S. aureus	10-14 (22.64)	11-18 (27.35)	26.5(50)
S. pyogenes	10-15 (23.58)	12-16 (25.92)	26.5(50)
Total	24.5(46.22)	28.5(53.78)	53

P≤0.05 , DZI: Diameter zone inhibition , Pearson Chi-Square = 0.074

Table (6): Antimicrobial effect of *Lactobacilli* spp. in millimeters using well diffusion method by cell free supernatant (CFS)

Isolates	Probiotic		Total No.(%)
	L .rhamnosus DZI by CFS (mm) No.(%)	*L. fermentum* DZI by CFS (mm) No.(%)	
S. aureus	6-12 (22.22)	8-13 (25.92)	19.5(48.14)
S. pyogenes	9-12 (25.92)	9-12(25.92)	21(51.86)
Total	19.5(48.14)	21(51.86)	40.5

P≤0.05, DZI: Diameter zone inhibition, Pearson Chi-Square = 0.105

The agar spot method yielded larger inhibition zones compared to the well diffusion method. *L. fermentum* produced an inhibition zone of 26 mm, while *L. rhamnosus* produced an inhibition zone of 22 mm. All inhibition zones were classified as highly active (≥12 mm). The results were statistically significant (*P* ≤ 0.001), as showed in Table (7)

Table (7): Antimicrobial effect of Lactobacilli spp. in millimeters using using agar spot method

Bacteria	**Probiotic**				**Total**
	L. rhamnosus		***L. fermentum***		
	Active ≥12mm (+++) No.(%)	Moderate active 7-12mm(++) No.(%)	Active ≥12mm(+++) No.(%)	Moderate active 7-12mm(++) No.(%)	No. (%)
S. aureus	10(62.5)	0(0%)	10(62.5)	0(0)	20(62.5)
S. pyogenes	6(37.5)	0(0%)	6(37.5)	0(0)	12(37.5)
Total	16(50)	0(0)	16(50)	0(0)	32

P≤0.001, Pearson Chi-Square = 6.33

Discussion

The development of antibiotic resistance is a threat to public health worldwide, and skin and soft tissue infections including impetigo are of particular concern. The current article aimed to report the contemporary antibiotic resistance profiles of *Staphylococcus aureus* and *Streptococcus pyogenes* associated with impetigo cases in Wasit, Iraq and investigates the potential of probiotics as a novel therapeutic application.

Our results, with *S. aureus* (48.0%) and then *S. pyogenes* (5.9%), as predominant pathogens, correlate well with the known burden of impetigo in the literature [1, 2]. The high prevalence of *S. aureus* highlights the need for efficacious anti-staphylococcal therapies. Furthermore, the age partition profile with the highest among children of 1–10 years (63.7%) also corresponds to the widespread occurrence of this pediatric disease [3].

The findings of antibiotic susceptibility observed in our study were disturbing and reflect an overall trend of emerging resistance in Iraq and Middle East [4, 5]. The very high resistance rate of *S. aureus* to penicillin G (90.3%) is not unexpected because penicillinase-producing *S. aureus* are now endemic [14]. But high resistance to the other frequently used antibiotics, erythromycin (35.4%) and azithromycin (29%), are clinically important. Current findings are also similar to few other recent studies from the same region. For example, in 2024 a review of *S. aureus* resistance rates in Iraq reported widespread resistance to penicillin and macrolides [4]. The studies in Pakistan and Nigeria revealed similar patterns of resistance as well [15, 16]. Erythromycin resistance in *S. aureus* is high (39.4%) among skin isolates from different 2025 skin infections in Greece [6]. We considered 100% sensitivity of our isolates towards levofloxacin and chloramphenicol to be good findings based on those results, raising interest in these agents for the treatment of blood stream infections. The high percentage (96.8%) vancomycin sensitivity observed could also still keep them effective as possibly useful therapeutic regimens against P.A. Nevertheless, isolation of even 1 VR isolate (3.2%) should be an early alarm in this regard and hence demands constant vigilant attention/scrutiny.

Secondly, the 100% penicillin G sensitivity seen for *S. pyogenes* is reassuring and in line with global experience, that of penicillin still being first-line antibiotic when treating infections caused by *streptococci* [7, 17]. However, 66.7% resistance to tetracycline and 33.3% emergence of resistance to ceftriaxone are worrying. Resistance to tetracycline for *S. pyogenes* has been well-documented and it has been rising however, in 2023, a study from Spain reported resistance of 12% [8] which is much lower than our findings, possibly reflecting local differences in resistance patterns. The high prevalence of tetracycline resistance in Iran and some other Middle Eastern countries has also been shown [21]. Resistance to ceftriaxone, a third generation cephalosporin is of particular concern given that such drugs are frequently used for more serious infections [19, 20]. The 100% sensitivity to linezolid seen in present study is in accordance with literature against Grampositive organisms [18].

An originality of our study consisted in the investigation of probiotic in vitro antibacterial activity against impetigo-related pathogens. It was found that both *Lactobacillus rhamnosus* and *Lactobacillus fermentum* had significant inhibitory effect against *S. aureus* and *S. pyogenes*. *L. fermentum* proved markedly more antibacterial than *L. rhamnosus* either in well diffusion or agar spot procedures. This result is supported by other studies, which have provided evidence for the antimicrobial effect of *Lactobacillus* species on skin pathogens [9, 10, 22, 23, 24].

The antibacterial mechanisms in which probiotics influence microbial metabolism are complex, and include the production of lactic acid, hydrogen peroxide, bacteriocin and biosurfactants, as well as competitive exclusion [11, 22]. Our findings indicated that BCB exerted more inhibitory effect than CFS, it was presumed that the living bacteria themselves and their secreted products contributed to the antimicrobial activity [24, 25]. The inhibition zones obtained by the agar spot method were significantly larger than the well technique, which suggests that this last had lower sensitivity for detection of antimicrobial activity, as reported elsewhere [12, 26].

The in vitro effectiveness of these probiotics implies they may serve as a useful substitute or supplement to antibiotics for impetigo treatment. Topical probiotics may contribute to the restauration of a normal skin microbiota, inhibit colonization by pathogenic bacteria and potentially limit selective pressure towards antibiotic resistance [9, 13, 27].

Limitations and Future Directions

This study has several limitations. First, it was a single-center study with relatively small population, the generalizability of our results is warranted to be verified. And second, the work was performed in a test tube and so the scientists have yet to prove that it really works for patients. Further studies of this topic should consist of multicenter, larger studies to validate our results and clinical randomized controlled trials assessing the effect and the safety of topical probiotics as treatment for impetigo. Furthermore, molecular examination of the resistant mechanisms within bacterial isolates would help obtain a better understanding on local epidemiology of antibiotic resistance.

Conclusion

This study demonstrated the large burden of antibiotic resistance affecting the treatment for impetigo in Wasit, Iraq, where high resistance levels were detected against many antibiotics among those commonly administered. The in vitro findings show the potential of *Lactobacillus rhamnosus* and *Lactobacillus fermentum* as novel antimicrobial agents for the primary impetigo pathogens. These results indicate the need for systematic monitoring of antibiotic resistance, also it warrants for additional research on probiotics as a safe and effective therapeutic alternative against skin infections, which will limit antibiotic resistance to inhibit bacteria that is close to that of antibiotics. Nevertheless, it is not dangerous in terms of side effects.

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