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Isolation of Acinetobacter bacteria from environmental samples in a university hospital

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Abstract:

Background: Hospital-acquired infections are considered one of the most confusing because of their association with antibiotic- and antiseptic-resistant bacteria. Acinetobacter bacteria are aerobic, Gram-negative coccobacilli, non- fermenting, isolated in hospital environments, causing infections that are widely spread in recent decades, and due to the spread of it in our hospitals causing hospital-acquired infections, this study will investigate it in peripheral samples from several wards in a University Hospital, with a comparison between the wards, and according to the type of taken sample.

Materials and methods: The study was conducted at Al-Mouwasat University Hospital in Damascus, on 50 samples taken from 5 wards: Intensive Care Unit, Fifth Aid, Burns and Plastic Surgery, General Surgery, and Internal medicine Ward. The taken samples were: air, water, patients' beds, mobile phones, resuscitation and breathing devices, and Acinetobacter were isolated in the microbiology laboratory between 1/1/2021 until 1/7/2021, and all Gram-positive, fermenting Gram-negative, and positive oxidase bacteria were excluded, identification is confirmed after performing Gram staining of the sample.

Results: Acinetobacter was isolated from 19 out of 50 samples (38%), and the largest positive percentage was from the Intensive Care unit (22%, 11/50), while it was not isolated from Internal Medicine ward, but for the samples, it was isolated for the largest proportion of Resuscitation equipment (6/50, 12%, and 6/19, 31.6%), while it was not isolated from water in all wards.

Conclusion: Our study confirmed the presence of Acinetobacter in the environment of the hospital, which is a source of infection for inpatients, while determining the difference in their spread among the wards of the hospital, in order to make the necessary recommendations to limit the spread and follow appropriate methods of disinfection and sterilization, in accordance with the nature of the studied sources of infection.

Key Words: Hospital-Acquired Infections, Acinetobacter Bacteria, Non-Fermenting Gram-Negative Bacteria.

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عزل جراثيم الراكدة من عينات محيطية في مشفى جامعي

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الملخص:

خلفية البحث وهدفه: تعتبر الإنتانات المكتسبة بالمشفى من الإنتانات الأكثر إرباكاً بسبب ارتباطها بجراثيم مقاومة للصادات والمطهرات، إضافة إلى وضع المريض الصحي الذي سبب دخوله للمشفى، والكلفة الاقتصادية المترتبة على العلاج.

الجراثيم الراكدة هي جراثيم مكورة عصوية سلبية الغرام هوائية مجبرة، غير مخمرة للسكريات، عزلت في بيئات المستشفيات مسببة انتانات منتشرة بشكل واضح في العقود الحديثة، ونظرا" لانتشارها في مشافينا مسببة إنتانات مكتسبة في المشفى، ستقوم هذه الدراسة بالتحري عنها لدى عينات محيطية من عدة شعب في مشفى المواساة الجامعي في دمشق، مع مقارنة بين الشعب، ومقارنة حسب نوع العينة المأخوذة.

مواد البحث وطرائقه: أجريت دراسة مقطعية مستعرضة في مشفى المواساة الجامعي، على 50 عينة مأخوذة من 5 شعب في المشفى هي: شعبة العناية المشددة، الإسعاف الخامس، الحروق والجراحة التجميلية، شعبة الجراحة العامة، وشعبة الداخلية، أما العينات المأخوذة فكانت عبارة عن: هواء، ماء، أسرة المرضى، هواتف جوالة، أجهزة الإنعاش والتنفس، وعزلت الراكدة في مخبر الأحياء الدقيقة في الفترة ما بين 2021/1/1 وحتى 2021/7/1، واستبعدت الجراثيم إيجابية الغرام كلها، والجراثيم سلبية الغرام المخمرة للسكاكر، وإيجابية اختبار الأوكسيداز، وأكد تحديد الهوية بعد إجراء تلوين غرام للعينة.

النتائج: عزلت الراكدة من 19 من 50 عينة مأخوذة (38% من العينات المعزولة إيجابية، وأكبر نسبة إيجابية كانت من شعبة العناية المشددة 22%، 50/11))، بينما لم تعزل من شعبة الباطنة، وبالنسبة للعينات، عزلت الراكدة بالنسبة الأكبر من أجهزة الإنعاش (50/6، 12%)، بينما لم تعزل من الماء في الشب الخمسة، ووجدت الراكدة في أجهزة الإنعاش في وحدة العناية المركزة بالنسبة الأكبر(19,31.6/6).

الاستنتاج: أكدت دراستنا وجود الجراثيم الراكدة في محيط المشفى، والتي هي مصدر للعدوى لدى المرضى المقيمين، مع تحديد اختلاف انتشارها بين شعب المشفى، مع اختلاف مصدر العدوى المحتمل، من أجل إجراء التوصيات اللازمة للحد من الانتشار واتباع طرق التطهير والتعقيم المناسبة، وبما يتناسب مع طبيعة مصادر العدوى المدروسة.

الكلمات المفتاحية: الإنتانات المكتسبة في المشفى، الجراثيم الراكدة، جراثيم سلبية الغرام غير المخمرة، أجهزة الإنعاش والتنفس.

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Introduction:

Acinetobacter bacteria is important pathogen that can cause a variety of health care-associated infections and iscontributed with morbidity and mortality. It is transmitted via contaminated hands, contact with contaminated surfaces or medical equipment, aspiration, inhalation, and also from patient to patient.(Gong *et al.*, 2016, p. 54)

Identification of Acinetobacter on environmental surfaces is important in clinical practice, especially during investigations of outbreaks, and in research to define modes of transmission.(McConnell *et al.*, 2012, p. 1412)

The genus Acinetobacter are defined as nonfermentative gram-negative coccobacilli, that are strictly aerobic, nonmotile, catalase positive and oxidase negative, their species play a significant role in the colonization and infection of inpatients.(Hussein *et al.*, 2013, p. 803)

They have been involved in a variety of nosocomial infections, including bacteremia, urinary tract infection, secondary meningitis, catheter-associated bloodstream, and agent of nosocomial pneumonia, particularly ventilator-associated pneumonia was confined to hospital intensive care units.(Constantiniu *et al.*, 2004, p. 35)

Acinetobacter nosocomial infections have become prevalent cause especially in immunocompromised and in Intensive Care Units (ICUs) patients in the last few years, and the bacteria has become an emerging pathogen especially in the hospitals owing to its ability to survive in adverse environmental conditions. (Gallego, 2016, p. 48)

Nosocomial outbreaks of Acinetobacter occur frequently. The survival of Acinetobacter in the environment plays an important role in the persistence of outbreaks, so it is necessary to eliminate the environmental sources of infection to control and stop the outbreaks.

The ability to gain multiple virulence factors, including resistance determinants such as motility, efflux pumps and iron acquisition mechanisms, help this bacterium to survive in adverse environmental conditions and facilitate the development of an infection. A propensity to tolerate drying and resistance to multiple classes of antibiotics are the key factors in enabling the organism to survive and spread in the nosocomial environment .(Al-Kadmy *et al.*, 2018, p. 51)

Acinetobacter is surviving as a commensal on the skin or hair of hospital staff and patients and colonizing a variety of body surfaces. (Pandey *et al.*, 2021, p. 2201) Various contaminated objects have been identified that serve as potential reservoirs for this nosocomial pathogen. There is evidence that in the hospital environment human infectious agents or items such as beds, tables, or gloves might carry infectious microorganisms.

In general, the treatment of infections with Acinetobacter is often extremely difficult because of the widespread resistance to the major groups of antibiotics of these species, (Raut *et al.*, 2020, p. 1631) So that the identification of the source or reservoir of Acinetobacter strains is very important to control future difficult infections and to curtail ongoing outbreaks.

Resistance of Acinetobacter to stress factors:

The emergence of nosocomial and community-acquired infections due to Acinetobacter are mainly a result of high adaptability to adverse environmental conditions and the ability to persist for months in dry and harsh environments, that many strains proliferate in moist environments and can survive for 5 to 11 months on dry surfaces and 60 min on fingertips. (Suleyman *et al.*, 2018, p. 20)

The resistance to desiccation facilitates its spread via hospital personnel, infrastructure, and medical devices.

Interestingly, up to 75% of hospitalized patients can become colonized with Acinetobacter spp. (Suleyman *et al.*, 2018, p. 20)

They are able to survive exposure to commonly used disinfectants such as chlorhexidine, and alcohol and are able to survive much better, compared with other gram-negative. (Harding *et al.*, 2018, p. 91)

Recent reports of the genetic requirements for Acinetobacter persistence identify virulence factors as zinc and iron acquisition systems, capsule and LPS biosynthesis genes, amino acid metabolism and acquisition genes, and the bfmRS TCS but more studies are needed to increase knowledge in these aspects.(Morris *et al.*, 2019, p. 1601)

Moreover, Acinetobacter is known to form biofilms both within the host. and also on abiotic surfaces such as hospital devices, which can further contribute to infection. Bacterial colonization in these biofilms increases extracellular stress tolerance and thus enhances bacterial persistence.(Gedefie *et al.*, 2021, p. 3711)

The ability of biofilm formation contributes to Acinetobacter easily survive and transfer in the hospital environment, such as attached to various

biotic and abiotic surfaces, (on fingertips and inanimate objects such as glass, plastic, vascular catheters, cerebrospinal fluid shunts or Foleys catheter) even after exposure to dry conditions and nutrient starvation during extended periods of time.(Narayanan et al., 2016, p. 847)

The ability to produce biofilms causes bacteria to endure relatively hard conditions. These features make eradication of biofilm-associated bacteria almost impossible from hospital environments. On the other hand, sensitivity to different antibiotics as well as microbial metabolism due to biofilm formation will be reduced. This is attributable to lack of food in the biofilm depth. Slower metabolism and antibiotic resistance lead to bacterial dissemination which can create a quick critical situation. This may increase the incidence of nosocomial infections caused by bacteria, especially in patients in intensive care and those in burn and surgery units.

Reference Studies:

Compared with global studies of stagnant prevalence in peripheral samples of hospitals in several countries around the world:

First, the air:

1-In a study in Liverpool in 1986, the Acinetobacter was isolated from the Intensive Care Unit and the Neurosurgery Division, and the prevalence rate was 16/82, 20%. Liverpool. (Allen & Green, 1987, p. 110)

2- A study in Hong Kong in 2000, the positivity rate was 53%. (Houang *et al.*, 2001, p. 228)

3- A study in the Miami Intensive Care Unit in 2013 showed acinetobacter bacteria in 12/53 or 22.6% of air samples. (Munoz-Price *et al.*, 2013, p. 1915)

4- While in another study in Miami but in 2015 for the intensive care unit, Acinetobacter in 21% of air samples. (Shimose *et al.*, 2015, p. 2346)

Second; the water:

1- A study in China in 2002 conducted in the intensive care unit was 0% water, that is, Acinetobacter was not isolated from the water.(Wang *et al.*, 2003, p. 102)

2- A 2012 Iranian study isolated Acinetobacter from 1.8% of water samples in the intensive care unit. (Yaslianifard *et al.*, 2012, p. 55)

3-While in another Iranian study in 2020, Acinetobacter was in 18% of the water samples. (Shamsizadeh *et al.*, 2020, p. 10)

Third; patients' beds:

1-In an Iranian study in 2017 of air, water and beds of patients in several hospitals, the presence of acinetobacter was found in 11% of air samples, 2% of water samples, and 17% of beds samples. (Shamsizadeh *et al.*, 2017, p. 250)

2- A Brazilian study in 2018 showed the presence of Acinetobacter in 23.7% of samples taken from ICU patients' beds in the hospital. (Rocha *et al.*, 2018, p. 438)

Forth; Mobile-phone:

1-Australian study in 2015 did not isolate Acinetobacter from 226 samples of mobile phones, i.e. 0%. (Chao Foong *et al.*, 2015, p. 12)

2-A study in Iran in 2017 conducted on 175 samples from mobile phones in the hospital, isolated Acinetobacter in 36.84% of the total samples. (Morubagal *et al.*, 2017, p. 143)

3- Indian study in 2018, isolated Acinetobacter by 12%, from 117 studied samples. (Siddiqui et al., 2020, p. 4.6)

4-A study in Zambia in 2021 had Acinetobacter in 2% of the samples studied on mobile phones.(Mushabati *et al.*, 2021, p. 259)

Fifth; Resuscitation and breathing equipment:

1- An Indian study in 2011 conducted swabs from resuscitation equipment in the intensive care unit of a children's hospital and 7/12 of the cultured samples were positive for acinetobacter, i.e. 58.3%. (Ebenezer *et al.*, 2011, p. 964)

2- A 2011 study in Taiwan isolated Acinetobacter from 27.3% (3/11) of samples taken from resuscitation equipment in the intensive care unit. (Huang *et al.*, 2011, p. 2211)

Aim:

Aim of study was to estimate the prevalence rate of isolates of Acinetobacter bacteria from various environmental samples, which were identification in microbiology laboratory at Al- Mouwasat University Hospita, In order to direct the necessary procedures to eliminate the sources of peripheral infection that cause hospital-acquired infections.

Place and Time of study:

The study was performed in the microbiology laboratory at Al-Mouwasat University Hospital, for six monthes in 2021.

The samples were collected from five wards of hospital: Intensive care unite, fifth aid ward, internal medicine ward, surgical ward, and burn and plastic surgery ward.

Materials and Methods:

Environmental samples were obtained from surfaces in hospital wards while occupied. Fifty samples

were collected (10 samples from each of the five enveromental media): air, water, patient beds, mobile-phones of medical staff, and resuscitation and breathing equipment.

Specimens collection

1-Air samples:

A total of 10 air samples were collected by putting media culture plate (MacConkey) at a height of 1.5 m above the ground level to simulate the breathing zone for 4 hours.

2-Water samples:

A total of 10 water samples were collected directly from the tap of water by sterilized swab, then inoculated on media culture plate (MacConkey)

3-Surface samples (beds, , mobile-phones of medical staff, and Resuscitation and breathing equipment):

A total of 30 surface samples were taken.

They were collected by the swab method, each site was sampled using a sterile cotton swab premoistened with normal saline; the swab was rolled back and forth over each surface three times to ensure that all sides of the swab made contact with the surface and that a maximal surface area was covered

After sampling, swab was placed into a sterile tube containing 2 mL of nutrient broth and was transferred to the laboratory

In the laboratory under aseptic conditions, the collected specimens were streaked directly on MacConkey agar for standard aerobic growth, incubated for 24 hrs at 37ºC. The colonies were 0.5-2 mm diameter, translucent to opaque (never pigmented), convex and entire opaque creamy and non lactose fermenting colonies on MacConkey agar were subcultured on Kligler iron agar (KIA) and incubated for another 24 hrs at 37ºC, the non-fermenting suger, and don't emit gas were performed oxidase test, then the negative oxidase bacteria (don't change the color of oxidase disc) were stained by Gram stain and examined by microscope, Acinetobacter appeared as tiny, Gram-negative coccobacillary cells (1-1.5X1.5-2.5µ) often appearing as diplococci.

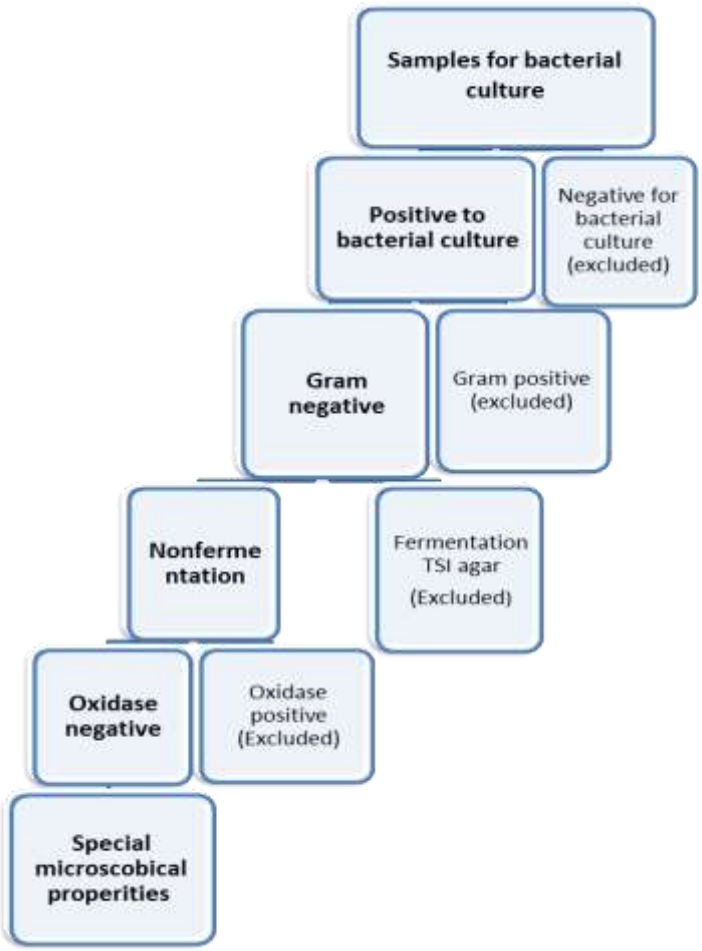

Figure (1) Identification of Acinetobacter

Statistical analysis:
The study used statistical program SPSS(Statistical package for social sciences).
P value was reported and a value of $P < 0.05$ was considered as a significant.

RESULTS:
The study was performed for 50 environmental samples (8 samples from each ward, except 18 samples from Intensive Care Unit) and the results were that: Out of 50 isolates, 19 isolates were positive for Acinetobacter (38%) and 31 were negative (62%).

1- A study of the distribution of acinetobacter samples by hospital wards: (table1)

(Table 1) prevalence of samples in the wards

Wards	Negative		Positive		Total
	Number	Rate	Rate	Number	
ICU*	7	38.9%	61.1%	11	18
Fifth aid	4	50%	50%	4	8
Burn & plastic surgery	5	62.5%	37.5%	3	8
Internal medicine	8	100%	0%	0	8
Surgical	7	87.5%	12.5%	1	8
Total	31	62%	38%	19	50

*Intensive Care Unit

In order to find out the statistical significance of the difference between sections in the percentage of positivity, Fisher's Exact Test was conducted, and it was:
p-value=0.014<0.05
Thus, there is a statistically significant difference between the departments in the percentage of transplant positivity.
The highest positive rate was in the intensive care unit (61.1%).

2-A study of the distribution of acinetobacter positive samples by hospital wards: (Table2)

(Table 2): Positive samples are distributed according to the wards

Wards	Positive	
	Numbers	Rate
Intensive Care Unit	11	57.9%
Fifth aid	4	21.1%
Burn & and plastic surgery	3	15.7%
Internal medicine	0	0%
General Surgical ward	1	5.3%
Total	19	100%

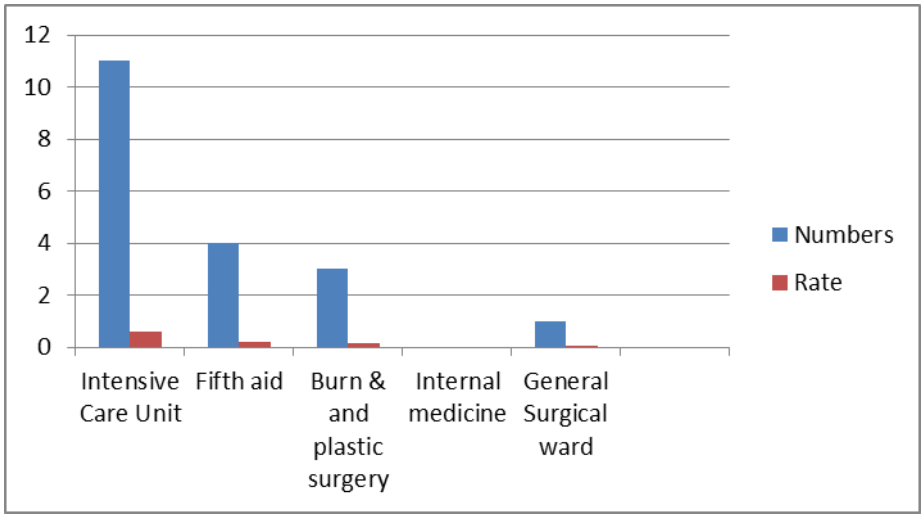

Figure (2): Positive samples are distributed according to the wards

3- A study of the distribution of positive samples according to the type of sample and the wadr from which the sample was taken: (Table 3)

Table (3): The distribution of positive cases according to the place where the sample was taken

Wards	Resuscitation and breathing equipment	Patients' bed	Mobile phones	Air	Water	Total
ICU	6 (54.5%)	2 (18.1%)	1 (9.1%)	2(18%)	0%	11 (100%)
Fifth aid	-	2 (50%)	1 (25%)	1(25%)	0%	4 (100%)
Burn & plastic surgery	-	1 (33.3%)	1 (33.3%)	1(33.3%)	0%	3 (100%)
Internal medicine	-	0 (0%)	0 (0%)	0(0%)	0%	0 (0%)
Surgical ward	-	0 (0%)	0 (0%)	1 (100%)	0%	1 (100%)
Total	6 (31.6%)	5 (26.3%)	3 (26.3%)	5(26%)	0%	19 (100%)

The highest positive rate was in the resuscitation equipment (60%)

4- A study of the distribution of positive and negative samples according to the type of sample: (Table 4)

Table (4): Distribution by sample type

Sampling location	Negative		Positive		Total
	Number	Rate	Number	Rate	
Water	10	100%	0	0%	10
Air	5	50%	5	50%	10
Mobile phone	7	70%	3	30%	10
Patients' bed	5	50%	5	50%	10
Resuscitation and breathing equipment	4	40%	6	60%	10
Total	31	62%	19	38%	50

The highest positive rate was in the resuscitation equipment (60%)

In order to find out the statistical significance of the difference between samples in the percentage of positivity, Fisher's Exact Test was conducted, and it was:

p-value=0.033<0.05

Thus, there is a statistically significant difference in the percentage of positive.

Discussion:

Previously, we reported the primary isolation rate, Acinetobacter are important infectious agent present in the hospital environment causing significant proportion of infections in specific patient wards, especially in critically ill patients in the ICU. Acinetobacter has emerged as an important nosocomial pathogen.

1-The study showed the presence of peripheral bacteria in samples taken from peripheral sources in the hospital, which is supposed to be at least one of the causes leading to acinetobacter hospital-acquired infections.

2-By studying 50 peripheral samples (8 samples from each department, except for the intensive care unit, 18 samples), and these peripheral samples were taken from 5 wards of the hospital, where acinetobacter is expected to appear more than others, and the prevalence rate was (38%, 19/50).), in all samples

3-The highest incidence of acinetobacter was in the Intensive Care Unit 57.9%, followed by the Fifth Aid Division 21.1%, while we had no positive peripheral samples in the Internal Medicine Division

4-In comparison between the type of sample studied and the division from which the sample was taken, the resuscitation and ventilator devices in the intensive care unit were the most contaminated with acinetobacter (6/19 = 31.6%) of all positive samples. Patients' beds and air were considered important factors in the process of transmission of infection, as each of them separately constituted 26.3% of the total positive samples, followed by mobile phones with a percentage of 15.8%, while water in our hospital did not constitute a cause of infection in any of the mentioned wards.

5-By comparing the spread of Acinetobacter according to the place of sampling, the resuscitation equipment had the largest percentage, and 60% of its samples showed positive implantation for acinetobacter bacteria, while air and patients' beds showed a positive rate of 50%.

6- The results in our study agreed with some results in international studies and were less than some of them and more than others in terms of prevalence. The results of the spread of Acinetobacter in the air in our hospital converged with the results of its spread in a study conducted in Hong Kong in 2000, while its spread in water was identical to a study conducted in China in 2002, and the beds of patients, the isolation of Acinetobacter was greater than the ratios in several international studies, and Acinetobacter was isolated from mobile phones at a rate close to that of an Iranian study in 2017, while the resuscitation equipment was close to the rates of isolation in an Indian study in 2011.

Conclusion:

The spread of Acinetobacter bacteria among hospitalized patients and in the hospital environment, and the high rate of morbidity and mortality associated with hospital-acquired infections, in addition to the increase in the economic burden resulting from this, makes the follow-up to the study of other sources of the presence of Acinetobacter obligatory, to give the necessary recommendations to eliminate it, in addition to repeat of study at intervals to ascertain the effectiveness of the methods used to get rid of them.

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