

## A Comparative Study of Bacterial Species Isolated from Diabetic Foot in Al-Najaf Governorate

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### Abstract:

**Background:** The World Health Organization defines diabetic foot as "a condition of infection, ulceration, or destruction of the deep tissues of the feet, associated with neuropathy and varying degrees of peripheral vascular disease in the lower limbs of diabetic patients." **Objective:** The aim of this study was to determine the prevalence of microorganisms causing infections in patients diagnosed with diabetic foot who were treated at Al-Sadr Educational Hospital in Najaf Governorate during the year 2024.

**Methodology:** This study involved isolating and diagnosing the main bacterial species responsible for the contamination of diabetic foot ulcers. A total of 118 swab samples were collected from diabetic patients hospitalized at Al-Sadr Educational Hospital. A descriptive, cross-sectional, and retrospective study was conducted from February 15 to December 15, 2024. A non-probability convenience sample was used for this study.

**Results:** It included 118 samples from diabetic patients reported as having diabetes. Regarding the distribution of participants by age groups, the highest results for women were in the age group 50-59 years, with 18 women, accounting for 29.50%. For men, the highest results were also in the same age group, with 17 men, accounting for 29.82%. As for the isolates, processing the isolates using a 3% potassium hydroxide solution resulted in the isolation of 87 Gram-negative isolates, constituting 73.7% of the total bacteria isolated. Additionally, 31 Gram-positive isolates were obtained, representing 26.3% of the total bacteria isolated in this study. Regarding the sensitivity of Gram-positive bacteria to antibiotics, the highest sensitivity was observed with Vancomycin, which showed 100% sensitivity for *Staphylococcus aureus*. Oxacillin also recorded a high sensitivity rate of 99% for the same type. **Conclusions:** The highest resistance was seen with Erythromycin, which showed 100% resistance for

*Enterococcus spp.* For the sensitivity of Gram-negative bacteria, the highest sensitivity was observed with Piperacillin, which showed 100% sensitivity for *Staphylococcus aureus*, indicating that this antibiotic is highly effective in treating infections caused by this type of bacteria. Imipenem also demonstrated good efficacy with 100% sensitivity towards *Klebsiella pneumoniae*. In contrast, Cefotaxime showed 0% sensitivity to *Staphylococcus aureus*, indicating it is completely ineffective against this type of bacteria. Similarly, Ampicillin recorded only 3.1% sensitivity for the same type.

**Keywords:** isolated bacteria, diabetic foot, Al-Najaf Al-Ashraf

## 1. Introduction

Diabetes mellitus (DM) is considered one of the major public health problems due to its high prevalence, morbidity, mortality, and the high cost of healthcare. It is defined as a condition in which the body fails to regulate blood glucose levels, caused by insufficient insulin production or ineffective insulin utilization. As a result, glucose cannot be properly absorbed, remaining in the bloodstream (hyperglycemia), which leads to tissue damage over time [1].

These pathological changes eventually lead to serious and potentially life-threatening complications. Diabetes is classified into three main types: Type 1 (DM1), Type 2 (DM2), and gestational diabetes. All are characterized by hyperglycemia, leading to severe acute and chronic complications such as macrovascular and microvascular diseases, myocardial infarction, stroke, kidney failure, blindness, peripheral neuropathy, and limb amputation [2]. Global statistics indicate that approximately 170 million individuals currently have diabetes, and this number is expected to double by 2030. Another statistic shows that about 15% of diabetic patients develop diabetic foot ulcers, commonly located under the heel [3]. These ulcers are a source of multiple complications such as infections in the nails, soft tissues, muscles, joints, tendons, and bone marrow. Deep tissue infections may induce immune dysfunction. Additionally, 84% of patients with such ulcers eventually undergo limb amputation [4].

Amputation and diabetic foot ulcers are among the most common complications in diabetic patients. The risk of lower-limb amputation increases nearly 40-fold compared to the general population. Mortality associated with immediate amputation is estimated

at 19%, while three-year survival is 65% and five-year survival is 41%. Diabetic foot occupies one of the highest ranks among global health problems, with an estimated 300 million affected individuals by 2025 [5].

The WHO defines diabetic foot as “a condition of infection, ulceration, or destruction of deep foot tissues associated with neuropathy and varying degrees of peripheral vascular disease among diabetic patients.” Diabetic foot infections are often caused by microorganisms belonging to *Staphylococcus spp.*, and to a lesser extent *Streptococcus spp.* More than 50% of infected ulcers contain Gram-negative aerobic and anaerobic bacilli, contributing to rapid and progressive wet gangrene, which may be fatal without proper treatment [6,7]. Rapid infection may present with subcutaneous swelling; however, this may also occur in diabetic patients infected with less virulent organisms such as *Escherichia coli* and other coliforms. The condition is more common in the elderly, although it increasingly affects younger adults and even children due to unhealthy lifestyles, including inactivity and poor diet [8].

Among children and adolescents, Type 1 diabetes is most common, with an incidence of 1.8 per 100,000 population, representing 28 to 30 new cases annually. This suggests a high likelihood of diabetic foot development as a complication [9]. Most diabetic patients have weakened immunity, increasing susceptibility to infections. Elevated blood glucose provides an ideal environment for bacterial proliferation, increasing the likelihood of chronic infections in diabetic foot ulcers, potentially leading to tissue necrosis and gangrene [9,10]. Common microorganisms involved in diabetic foot infections include *Pseudomonas spp.*, *Staphylococcus aureus*, *E. coli*, *Proteus spp.*, *Enterobacter spp.*, and *Clostridium perfringens*. The prevalence of wound infections varies by region, complicating wound healing and necessitating specialized healthcare [11].

## 2. Materials and Methods

### 2.1 Collection of Diabetic Foot Ulcer Samples

To isolate microorganisms responsible for diabetic foot ulcer infections, 118 cotton swab samples were collected from diabetic patients hospitalized at Al-Sadr Teaching Hospital. A descriptive, cross-sectional, retrospective study was conducted between February 15 and December 15, 2024. A non-probability convenience sampling

technique was adopted. Samples were cultured on MacConkey agar, 5% sheep blood agar, and chocolate agar using the streak-plate method. Identification to genus and species was performed using conventional biochemical tests. Gram-negative bacteria were identified using oxidase, TSI, citrate, ornithine, lysine, SIM, urea, and phenylalanine tests.

Gram-positive bacteria were identified using catalase, coagulase, Bile Esculin, Staphylococcus aureus latex test, ornithine, and polymyxin B. Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method. Gram-positive bacteria were tested against Ciprofloxacin, Clindamycin, Erythromycin, Cefoxitin, Rifampicin, and Vancomycin. Gram-negative bacteria were tested against Ampicillin, Ceftazidime, Amoxicillin/Clavulanic acid, Cefotaxime, Cefepime, Ciprofloxacin, Imipenem, Meropenem, Piperacillin, and Colistin.

### 2.1.2 Culture Media Used

**Blood Agar Base:** 40 g/L dissolved, sterilized, cooled to 45–50°C, then supplemented with 5% human blood. Used to detect hemolysis.

### 2.1.3 Additional Media

**Blood Agar Base** (51.5 g/L): Used to isolate bacteria and detect lactose fermentation.

**Mannitol Salt Agar** (108 g/L): For isolating staphylococci and detecting mannitol fermentation.

**Nutrient Broth** (13 g/L): For bacterial activation and growth.

**Muller-Hinton Agar** (38 g/L): For antibiotic susceptibility testing.

**Brain-Heart Infusion Broth:** For bacterial culture and long-term preservation with glycerol.

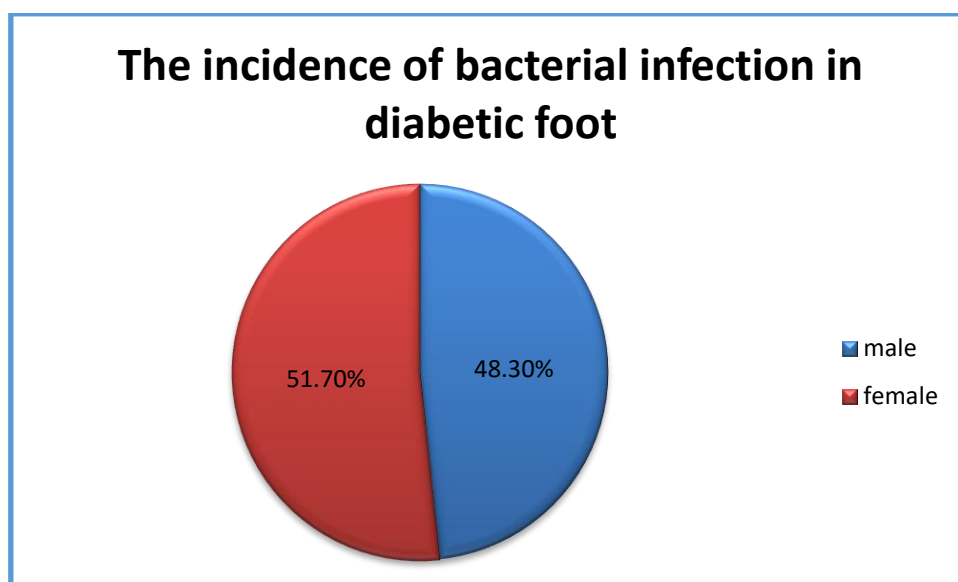
## 3. Results

### 3.1 Age and Sex Distribution

The highest percentage among females was in the age group **50–59 years** (29.50%), and similarly for males (29.82%). The lowest percentage among males was in the age group **80–89 years** (5.2%), while among females the lowest was **20–29 years** (3.27%).

**Table 1:** Percentage distribution of bacterial infections in diabetic foot according to age group and sex

Age groups	(%)	No.	Female		Male	
			(%)	No.	(%)	No.
29-20	6.77	8	3.27	2	10.52	6
39-30	9.32	11	8.19	5	10.52	6
49-40	19.49	23	21.31	13	17.54	10
59-50	29.66	35	29.50	18	29.82	17
69-60	16.10	19	8.9	9	17.54	10
79-70	11.0	11	14.75	6	8.77	5
89-80	9.3	8	8.19	5	5.2	3
90 <	2.5	3	4.91	3	-	-
<b>Total</b>	<b>100</b>	<b>118</b>	<b>118</b>	<b>61</b>	<b>100</b>	<b>57</b>



**Figure (1)** The distribution pattern affecting diabetic patients according to gender

### 3.1 Isolation and Identification of Bacteria

After culturing samples taken from diabetic foot ulcers on blood agar and MacConkey agar, 118 bacterial isolates were obtained. Treatment of the isolates with 3% potassium hydroxide solution resulted in the isolation of 87 Gram-negative isolates, representing 73.7% of the total bacteria isolated. Additionally, 31 Gram-positive isolates were obtained, representing 26.3% of the total bacteria isolated in this study. The table shows that *\*Enterococcus spp.\** were the most common Gram-positive

bacteria, with 25 isolates (28.74%). In contrast, *Streptococcus viridians*\* were the least common, with 4 isolates (%4.60) .As for the Gram-negative bacteria, *Acinetobacter spp.\** were the most prevalent. The list includes 9 bacteria (29.03%), while *Enterobacter cloacae*, *Providencia alcalifaciens*, and *Enterobacter spp.* share the lowest ranking, with 1 bacterium each (3.23%). Overall, there is a clear predominance of positive bacteria compared to negative bacteria.

**Table 2:** Bacterial species isolated from diabetic foot ulcers in Al-Najaf

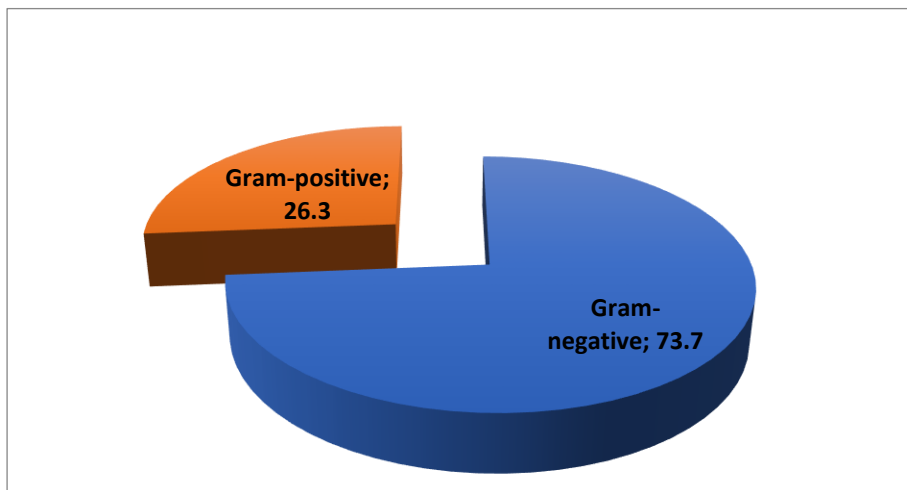
Gram-positive bacteria	No.	%
<i>Enterococcus spp.</i>	25	28.74
<i>Staphylococcus aureus</i>	14	16.09
<i>Staphylococcus coagulase (-)</i>	11	12.64
<i>Streptococcus group "A"</i>	9	10.34
<i>Staphylococcus epidermidis</i>	7	8.05
<i>Enterococcus faecalis</i>	6	6.90
<i>Streptococcus spp.</i>	6	6.90
<i>Streptococcus agalactiae</i>	5	5.75
<i>Streptococcus viridians</i>	4	4.60
<b>Total</b>	<b>87</b>	<b>100</b>
Gram-negative bacteria	No.	%
<i>Acinetobacter spp</i>	9	29.03
<i>Pseudomonas aeruginosa</i>	6	19.35
<i>Klebsiella pneumoniae</i>	4	12.90
<i>Pseudomonas spp.</i>	3	9.68
<i>Escherichia coli</i>	2	6.45
<i>Proteus vulgaris bilis</i>	2	6.45
<i>Proteus mira</i>	2	6.45
<i>Enterobacter cloacae</i>	1	3.23
<i>Providencia alcalifaciens</i>	1	3.23
<i>Enterobacter spp</i>	1	3.23
<b>Total</b>	<b>31</b>	<b>100</b>

### 3.2 Antibiotic Sensitivity

#### 3-2-1 Antibiotic Sensitivity of Gram-Positive Bacteria:

Table 3 shows the antibiotic sensitivity of Gram-positive bacteria isolated from the feet of diabetic patients. The table includes three main types of bacteria: *Staphylococcus*

*aureus*, *Enterococcus spp.*, and *Group A Streptococcus*. The highest sensitivity was observed with vancomycin, at 100% for *Staphylococcus aureus*. Oxacycline also showed a high sensitivity of 99% for the same type. The highest resistance was observed with erythromycin, at 100% for *Enterococcus spp.*, meaning that this bacterium was completely unaffected by the antibiotic. The lowest sensitivity was observed with levofloxacin, at only 33% for *Staphylococcus aureus*. Overall, the table



demonstrates the importance of selecting appropriate antibiotics based on bacterial sensitivity and resistance, which helps in providing effective treatments for patients.

**Figure (2) Percentage of Gram-positive and Gram-negative bacteria**

**Table 3:** Antibiotic susceptibility of Gram-positive bacteria isolated from diabetic foot patients

<i>Streptococcus Group A</i>				<i>Enterococcus spp.</i>				<i>Staphylococcus aureus</i>				Antibiotics
R		S		R		S		R		S		
%	N	%	N	%	N	%	N	%	N	%	N	
-	0	100	4	0	0	100	6	-	-	-	-	Erythromycin
0	0	100	4	35,3	2	66,6	4	9,1	3	89,6	21	Ampiciline
100	0	100	4	-	-	-	-	22,7	7	73,2	18	Ciprofloxacin
0	4	0	0	-	-	-	-	18,1	4	71,8	10	Clindamycin
100	-	-	-	-	-	-	-	0	0	100	31	Vancomycin
0	-	-	-	-	-	-	-	100	19	33	7	Levofloxacin
0	-	-	-	-	-	-	-	0	0	99	24	Oxacycline
100	-	-	-	0	-	100	6	0	0	97	20	Rifampicin

### 3-2-2 Antibiotic Sensitivity of Gram-Negative Bacteria:

Table (4) shows the results of antibiotic resistance for three types of bacteria: *Klebsiella pneumoniae*, *Acinetobacter spp.*, and *Pseudomonas aeruginosa*. The table shows the percentage of bacteria that were sensitive (S) or resistant (R) to a range of antibiotics, along with the number of samples for each type. For cefotaxime, no sensitivity was shown by *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*, while resistance was 100% in *Acinetobacter spp.* Ampicillin showed low sensitivity with *Klebsiella pneumoniae* (3.1%) and *Acinetobacter spp.* (10.1%), while all *Pseudomonas aeruginosa* samples were resistant.

Cefepime showed mixed results, with a sensitivity rate of 5% for *Klebsiella pneumoniae* and 11% for *Acinetobacter spp.*, while resistance was 68.7%. Ciprofloxacin showed sensitivity of 9.8% for *Klebsiella pneumoniae* and 6.1% for *Acinetobacter spp.*, with notable resistance in *Pseudomonas aeruginosa* (3%). Imipenem showed 16% sensitivity to *Klebsiella pneumoniae*, while all samples of *Acinetobacter spp.* were resistant. Piperacillin achieved good results with *Klebsiella pneumoniae* (100% sensitivity), while resistance in *Acinetobacter spp.* was 61.4%. Meropenem showed moderate sensitivity with 3% for *Klebsiella pneumoniae*, while all *Pseudomonas aeruginosa* were sensitive. Finally, piperacillin/tazobactam showed 5% sensitivity to *Klebsiella pneumoniae* and 18% resistance to *Acinetobacter spp.* Overall, the table illustrates significant challenges in treating infections caused by these bacteria, with a high rate of resistance to multiple antibiotics.

**Table 4:** Antibiotic sensitivity of Gram-negative bacteria isolated from diabetic foot patients

<i>Pseudomona aeruginosa</i>				<i>Acinetobacter spp.</i>				<i>Klebsiella pneumoniae</i>				Antibiotics
R		S		R		S		R		S		
%	N	%	N	%	N	%	N	%	N	%	N	
100	-	-	-	100	14	0	0	100	16	0	0	Cefotaxime
-	-	-	-	100	14	0	0	63,3	10,1	46,1	3,1	Ampiciline
22,1	5,5	57,1	7,4	71,4	10	28,5	4	68,7	11	31,2	5	Cefepime
18,1	3,7	71,4	9,2	78,6	11	21,4	3	38,4	6,1	51,5	9,8	Ciprofloxacin
25,4	4,6	64,2	8,3	42,8	6	77,1	8	100	13	0	16	Imipenem
0	0	100	13	28,5	4	61,4	10	0	0	100	16	Piperacycline

28,5	3,7	71,4	9,2	100	14	0	0	51,2	15	18,7	3	Meropenem
40	3,5	40	6,5	48,5	11	21,4	3	78,1	18	21,2	5	Piperazilin / Tazo

**4 – Discussion:**

The study shows a 51% prevalence of bacterial infections among women. The participant distribution indicates that the 50-59 age group has the highest representation, at 29.50% among women and 29.82% among men. This phenomenon aligns with the findings of study [12], which found that chronic diseases, such as diabetes and heart disease, are more common in this age group. This concentration is attributed to factors such as physiological changes associated with aging, lifestyle, and psychological stress, which are more prevalent at this stage.

In contrast, the least represented age groups were 20-29 years for women and 80-89 years for men. This lower representation among younger women may reflect a range of socioeconomic factors, such as access to healthcare, lack of health awareness, or even differences in social activities. The lower representation among older adults may be attributed to age-related health factors, such as high mortality rates or poor health status, which affect their ability to participate in studies [13,14]. Furthermore, the study [15] indicates that older age groups often suffer from multiple diagnoses, which may lead to an inability to participate in research. It is also important to note that these findings highlight the urgent need to develop public health strategies targeting different age groups, especially underrepresented ones, to ensure the inclusivity of future research and studies. [16]

Culturing samples taken from diabetic foot ulcers on blood agar and MacConkey agar reveals a significant diversity of bacterial isolates. A total of 118 bacterial isolates were obtained. Treatment with 3% potassium hydroxide solution showed that 73.7% of the isolates were Gram-negative, while 26.3% were Gram-positive, highlighting the general tendency towards Gram-negative bacteria in this clinical presentation [17].

The results suggest that Enterococcus spp. It is the most common Gram-positive bacterium, consistent with previous studies such as those conducted by [18], which have reported its frequent presence in skin and soft tissue infections. This is attributed to Enterococcus' ability to adapt and grow in diverse environments, making it a

common pathogen in diabetic patients. On the other hand, negative isolate results show that *Acinetobacter spp.* is the most common, which aligns with research indicating that this bacterium is often isolated from wounds and ulcers in immunocompromised patients. *Acinetobacter* is considered a multidrug-resistant microorganism, posing significant treatment challenges and necessitating attention to effective control strategies [19,20].

The results also indicate that Gram-positive bacteria such as *\*Enterobacter cloacae\** and *\*Providencia alcalifaciens\** were underrepresented, which may reflect the specific infectious nature of diabetic foot ulcers. According to the study (name of study), environmental and clinical factors can complicate the infection, explaining the variability in the isolated bacterial patterns. Overall, these findings highlight the clinical challenges associated with managing diabetic foot ulcers, where the predominance of Gram-positive bacteria is evident, warranting further research to understand the factors influencing the prevalence of these bacterial species. Investigating the relationships between these isolates and their pathological characteristics could contribute to improving treatment and prevention strategies for patients in the future [20,21]. The results of the antibiotic susceptibility study of Gram-positive bacteria isolated from diabetic foot ulcers are of great importance in guiding treatment strategies.

The main bacterial species isolated were *\*Staphylococcus aureus\**, *\*Enterococcus spp.\**, and *\*Streptococcus Group A\**, which exhibited a diverse response to antibiotics. The results showed that vancomycin was the most effective, exhibiting 100% sensitivity against *Staphylococcus aureus*. This aligns with study [22], which confirmed vancomycin's efficacy as a primary treatment for *Staphylococcus aureus* infections, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Oxacillin also demonstrated a high sensitivity rate of 99% against the same strain, reflecting the effectiveness of these antibiotics in controlling bacterial infections [23].

On the other hand, the results showed a remarkable resistance of *Enterococcus spp.* bacteria to erythromycin, at 100%, indicating the ineffectiveness of this antibiotic against these bacteria. This aligns with studies [24,25] that warned of the spread of erythromycin resistance among *Enterococcus*, reflecting the need to reassess the use of this antibiotic in treatment. As for the lowest sensitivity rate, it was observed with levofloxacin at 33% for *Staphylococcus aureus*, indicating a decline in the effectiveness

of this antibiotic in treating infections caused by this type of bacteria. This reflects the need for alternative treatment strategies, especially given the increasing prevalence of resistance. Overall, these results highlight the importance of selecting antibiotics based on bacterial susceptibility and resistance analysis. Understanding resistance patterns in bacteria can help improve the treatments prescribed for patients, thereby reducing treatment failure rates and infection-related complications. Therefore, the pursuit of accurate and reliable susceptibility testing should be an essential part of managing bacterial infections in diabetic patients [26]. Antibiotic resistance is a critical issue in modern medicine, leading to the failure of conventional treatments and increased mortality rates.

The table shows resistance in three bacterial species: *Klebsiella pneumoniae*, *Acinetobacter spp.*, and *Pseudomonas aeruginosa*. Study [27] demonstrated that *Klebsiella pneumoniae* has developed high levels of resistance to several antibiotics, including cefotaxime and ampicillin., resistance to cefotaxime reached up to 70% in some clinical settings, consistent with the 0% sensitivity reported in the current table. Imipenem is considered the most effective option, as research suggests its use can improve treatment outcomes, but it may be associated with increased treatment costs. *Acinetobacter spp.* *Pseudomonas aeruginosa* is a bacterium known for its high resistance, and studies have shown that it is capable of developing multiple resistance mechanisms, including the production of  $\beta$ -lactamase enzymes [28].

The current findings, which demonstrate 100% resistance to cefotaxime, are consistent with research highlighting high levels of resistance in clinical settings. This analysis underscores the importance of developing novel strategies, such as the use of combination therapy, which has shown efficacy in some studies [29]. *Pseudomonas aeruginosa* is a common bacterium associated with hospital-acquired infections and is known for its high antibiotic resistance. This bacterium exhibits resistance to multiple antibiotics but may respond to certain antibiotics such as piperacillin and meropenem. The results in the table, which show complete sensitivity to piperacillin, support this trend, making it a potential treatment option.

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