

Prevalence of Extended-Spectrum β -Lactamases and Antibiotic Resistance Patterns in *Acinetobacter baumannii* Isolated from Clinical Samples in Kermanshah, Iran

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Abstract

Background: *Acinetobacter* is an opportunistic bacterial pathogen with intrinsic and acquired resistance to many antibiotics.

Objectives: This study aimed to determine the prevalence of extended-spectrum β -lactamases (ESBLs) and antibiotic resistance patterns in *A. baumannii* isolated from clinical samples at Imam Reza hospital in Kermanshah, Iran, using phenotypic and genotypic methods.

Methods: In this descriptive-analytical study, 80 *Acinetobacter* isolates obtained from clinical samples were confirmed using standard biochemical tests. After antibiotic susceptibility testing using disk diffusion method, the presence of ESBLs was detected using the combined disk test (CDT). The Polymerase chain reaction (PCR) method was employed to identify the frequency of *bla*TEM, *bla*CTX-M, and *bla*SHV genes using their specific primers.

Results: Among the *A. baumannii* isolates, 62.5% showed multidrug resistance (MDR). The highest rate of antibiotic resistance was to ceftriaxone (100%), followed by amikacin (96.2%), and the lowest was to polymyxin B (13.8%) and ampicillin/sulbactam (52.8%). Forty-three (53.8%) ESBL-producing isolates expressing *SHV* (n = 18, 41.9%), *TEM* (n = 11, 25.6%), and *CTX-M* (n = 3, 7%) genes were identified.

Conclusions: This study revealed an increased prevalence of ESBL-encoding genes in *A. baumannii* isolates. The increased frequency of these genes may be due to overuse or inappropriate use of antibiotics in this region. To combat overuse and prevent further development of drug resistance in Kermanshah, Iran, more attention should be paid to prescribing practices for various antibiotics, including cephalosporins.

Keywords: Extended-Spectrum Beta-Lactamase (ESBL), Antibiotic Resistance, Multidrug Resistance, *Acinetobacter baumannii*

1. Background

Acinetobacter is a Gram-negative, polymorphic, non-motile, strictly aerobic bacterium, which can be easily grown in a conventional laboratory environment (1). Different species of *Acinetobacter* cause urinary tract infections, wound infections, meningitis, endocarditis, peritonitis, and dermal and soft tissue infections (2). This opportunistic bacterium can survive on various surfaces, including patient's skin and used medical equipment. Thus, prolonged hospitalization in the intensive care unit, severe disease, immune system deficiency, burn injuries, long-term use of antimicrobial agents, and catheter use are risk factors for *Acinetobacter* infection (3).

Acinetobacter baumannii is more prevalent than other

strains of *Acinetobacter*, and it is the most common cause of bacterium-induced infections. To treat infections caused by *Acinetobacter*, various antibiotics, including β -lactams, fluoroquinolones, and aminoglycosides, are used; however, inappropriate and indiscriminate use of these antibiotics in recent years has led to the emergence of strains that are highly resistant to antimicrobial agents (4). Such resistance can be innate or caused by genetic resistance factors (5). Multidrug resistance (MDR) of *Acinetobacter* has been reported (6). The production of β -lactamase enzymes is a major mechanism of antimicrobial resistance and a major problem in the treatment of various infectious diseases. These enzymes can disable β -lactam antibiotics by hydrolysis of the central core of the antibiotics. Of vari-

ous β -lactamases, ESBLs have greater functions (7). The vast majority of ESBLs are the result of mutations in *CTX-M*, *SHV* (sulphydryl variable), and *TEM* (Temoneira) genes (8).

With the increase in drug resistance, ESBLs limit the choice of common antibiotics that can be used. Drug resistance can be caused by the transfer of resistant genes carried on plasmids and simultaneous transfer of other antibiotic-resistance genes (9). In some cases, due to high resistance to common antibiotics, ESBL-producing bacteria can lead to mortality by disrupting the treatment of infectious diseases (10). Given the high prevalence of hospital infections, studies on the causative microorganisms, including their antibiotic resistance patterns, are of paramount importance.

2. Objectives

There are no recent comprehensive studies in Kermanshah on the prevalence of *CTX-M*, *TEM*, and *SHV* genes in *A. baumannii*. Thus, the aim of the present study was to determine the prevalence of extended-spectrum β -lactamases (ESBLs) and antibiotic resistance patterns in *A. baumannii* isolated from clinical samples at Imam Reza hospital in Kermanshah, Iran, using phenotypic and genotypic methods.

3. Methods

3.1. Ethics Statement

The ethics committee of Kermanshah University of Medical Sciences approved the study protocol (No.: 1394.250).

3.2. Bacterial Isolates and Identification

In this descriptive cross-sectional study, 385 clinical samples were collected from different sources (endotracheal tubes, blood, sputum, urine, catheters, wounds, and various liquids) at Imam Reza hospital in Kermanshah from January 2016 to November 2016. Following collection, the samples were transported to the laboratory, cultured on blood agar and MacConkey agar (Merck, Germany), and then incubated for 24 hours at 37°C. In total, 80 isolates of *A. baumannii* were identified and confirmed using standard biochemical tests, including growth on citrate media and nonfermentable lactose, oxidase and catalase activity, immobility on SIM medium, ALK/ALK patterns on TSI medium (Himedia CO, India), and lack of pigment production. The PCR method using *OXA-51* gene was used for final confirmation of the *Acinetobacter* isolates (11).

3.3. Antibiotic Susceptibility Testing

The antibiotic resistance of the isolates was determined using Kirby–Bauer disk diffusion method, according to the guidelines of the clinical and laboratory standards institute (CLSI) (12). To this end, 14 antibiotic discs (MAST, U.K.) were used: gentamicin (10 μ g), amikacin (30 μ g), tobramycin (10 μ g), imipenem (10 μ g), meropenem (10 μ g), ceftazidime (30 μ g), cefotaxime (30 μ g), ceftriaxone (30 μ g), ciprofloxacin (5 μ g), levofloxacin (5 μ g), piperacillin/tazobactam (10/100 μ g), ticarcillin/clavulanic acid (10/75 μ g), ampicillin/sulbactam (20 μ g), and polymyxine B (300 μ g). The *A. baumannii* suspension was spread on Mueller-Hinton agar medium (Himedia CO, India) after incubation at 37°C and compared with a 0.5 McFarland standard using the lawn culture method. The disks were then placed on them. After incubation in an incubator for 24 hours, the growth inhibition diameter was measured and compared with that listed in CLSI tables. *Escherichia coli* ATCC 25922 was used for quality control of the reaction. *Acinetobacter* isolates showing resistance to three or more antibiotic strains were regarded as MDR strains.

3.4. Amplification of *blaTEM*, *blaSHV*, and *blaCTX-M* Genes by the PCR Method

To identify ESBL-producing isolates, the combined disk method was used. First, a standard concentration of *A. baumannii* was lawn cultured on Mueller-Hinton medium. Then, discs containing ceftazidime, ceftazidime/clavulanic acid, cefotaxime, and cefotaxime/clavulanic acid were placed 15 mm away from each other on the plates. After incubation for 24 hours at 37°C, the zone of inhibition around the disk containing clavulanic acid only was compared to that of the same ESBL disk as the enzyme inhibitor was measured. If the zone of inhibition around the disk containing clavulanic acid was greater than or equal to 5 mm as compared to that of the same disk without clavulanic acid, the strain was considered an ESBL-producing strain in accordance with CLSI guidelines. A standard *Escherichia coli* strain, ATCC 25922, was used as positive control. For PCR, DNA was extracted from the isolates using boiling method, and the frequencies of *SHV*, *TEM*, and *CTX-M* genes were investigated using specific primers (Takapou Zist Co., Iran), as shown in Table 1 (13, 14).

3.5. PCR Assay

The PCR assay was conducted using 12.5 mL Master Mix (SinaClon Company, Iran), 1 μ g of each primer, 3 μ g bacterial DNA, and distilled water to obtain a final volume of 25 mL. Except for the annealing temperature, the PCR reaction temperature was the same for all the three β -lactamase genes. It consisted of initial denaturation for

Table 1. The Nucleotide Sequence of Primers Used in the Detection of ESBL-Producing *A. Baumannii*

Gene	Nucleotide Sequence (5' -3')	Annealing, °C	Product Size, bp
<i>blaSHV</i>	5'-CTTACTCGCTTTATCG-3'	53	868
	5'-TCCCGCAGATAAATCAC-3'		
<i>blaTEM</i>	5'-GAGTATTCAACATTCCGGTGC-3'	45	800
	5'-TAATCAGTGAGGCACCTATCTC-3'		
<i>blaCTX-M</i>	5'-ATGTGCAGTACCAGTAAGGT-3'	53	594
	5'-TGGGTAAAGTAGGTCACCAGA-3'		

4 minutes at 94°C, followed by 35 cycles of denaturation for 1 minute at 94°C, amplification at 72°C for 40 seconds, and final amplification at 72°C for 5 minutes (Table 1). Finally, the PCR products were examined using electrophoresis and staining with ethidium bromide.

3.6. Statistical Analysis

Chi-square and Fisher's tests were conducted. All statistical analyses were performed using statistical package for the social sciences (SPSS), version 20.

4. Results

In the 385 clinical samples examined in this study, 80 (20.8%) isolates of *A. baumannii* were detected. In the study, 43 (53.8%) samples were obtained from males, and 37 (46.2%) from females, with a mean age of 48.38 ± 14.7 , ranging from 19 to 82 years. Majority of the isolates were detected in respiratory samples ($n = 40$, 50%). The lowest detection frequency was in cerebrospinal fluid and pleural fluid ($n = 4$, 5%) (Figure 1).

As shown by the antibiotic resistance patterns of *A. baumannii* given in Table 2, the highest resistance was observed against ceftriaxone (100%) and amikacin (96.2%), whereas the lowest resistance was against polymyxin B (13.8%) and ampicillin/sulbactam (52.8%). Among the isolates, 50 (62.5%) showed MDR against the antibiotics tested. Based on the results of the confirmatory combined-disk test, 43 (53.8%) of the 80 *A. baumannii* isolates showed ESBL production. The frequency of ESBL genes in the clinical samples is shown in Figure 1. ESBL genes were not significantly correlated with the age and gender of the patients ($P > 0.05$).

18 (41.9%), 11 (25.6%), and 3 (7%) ESBL-producing isolates expressed *SHV*, *TEM*, and *CTX-M* β -lactamase genes, respectively. The frequency of ESBL genes in the MDR *Acinetobacter* isolates according to the type of clinical sample is shown in Table 3. There was a statistically significant association between ESBL genes and resistance to some antibiotics. For example, there was a significant relationship

Table 2. Results of Antibiotic Resistance of *A. Baumannii* Strains Isolated from Clinical Specimens^a

Antibiotic	<i>A. Baumannii</i> Isolated		
	Resistant	Intermediate	Susceptible
Ceftazidime	76 (95)	0	4 (5)
Ceftriaxone	80 (100)	0	0
Cefotaxime	73 (91.2)	1 (1.2)	6 (7.5)
Ciprofloxacin	64 (80)	4 (5)	12 (15)
Levofloxacin	57 (71.2)	10 (12.5)	13 (16.2)
Gentamicin	63 (78.8)	2 (2.5)	15 (18.8)
Amikacin	77 (96.2)	0	3 (3.8)
Tobramycin	54 (67.5)	4 (5)	22 (27.5)
Imipenem	68 (85)	2 (2.5)	10 (12.5)
Meropenem	64 (80)	2 (2.5)	14 (17.5)
Ampicillin/sulbactam	42 (52.5)	13 (16.2)	25 (31.2)
Piperacillin/tazobactam	58 (72.5)	3 (3.8)	19 (23.8)
Ticarcillin/clavulanic acid	55 (68.8)	5 (6.2)	20 (25)
Polymyxin B	11 (13.8)	6 (7.5)	63 (78.8)

^aValues are expressed as No. (%).

between the expression of *SHV* gene and resistance to cefotaxime ($P = 0.038$) and tobramycin ($P = 0.002$). In addition, the expression of *TEM* gene was associated with resistance to piperacillin/tazobactam ($P = 0.021$), and the expression of *CTX-M* gene was associated with resistance to levofloxacin ($P = 0.05$). The results of PCR assay are given in Figure 2.

5. Discussion

In this study, similar to studies by Farajnia in Tabriz, Iran, and Sinha in India, the majority of isolates of *A. baumannii* were detected in respiratory samples (15, 16). In contrast, in a study by Vazirzadeh et al., isolates were

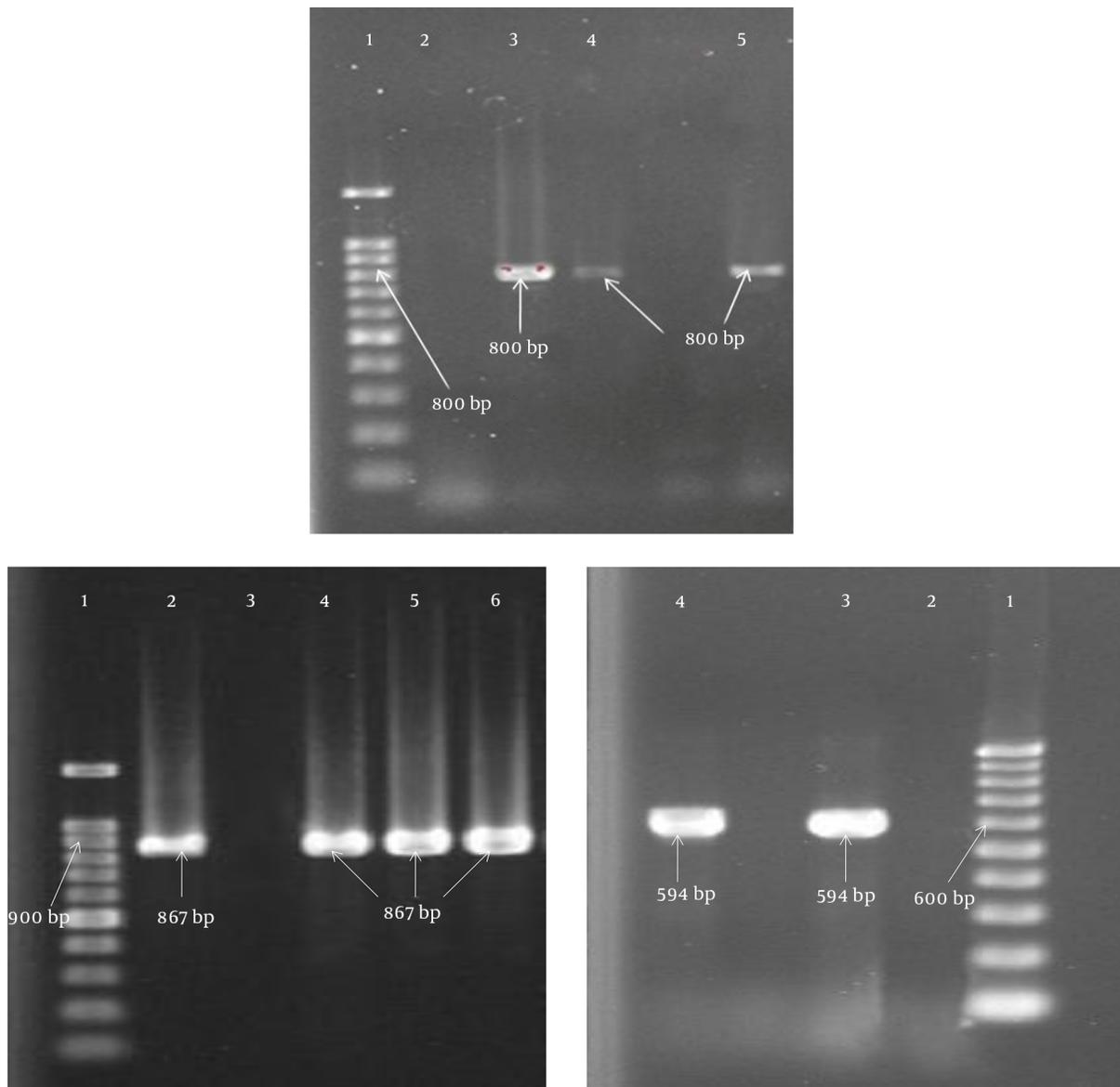


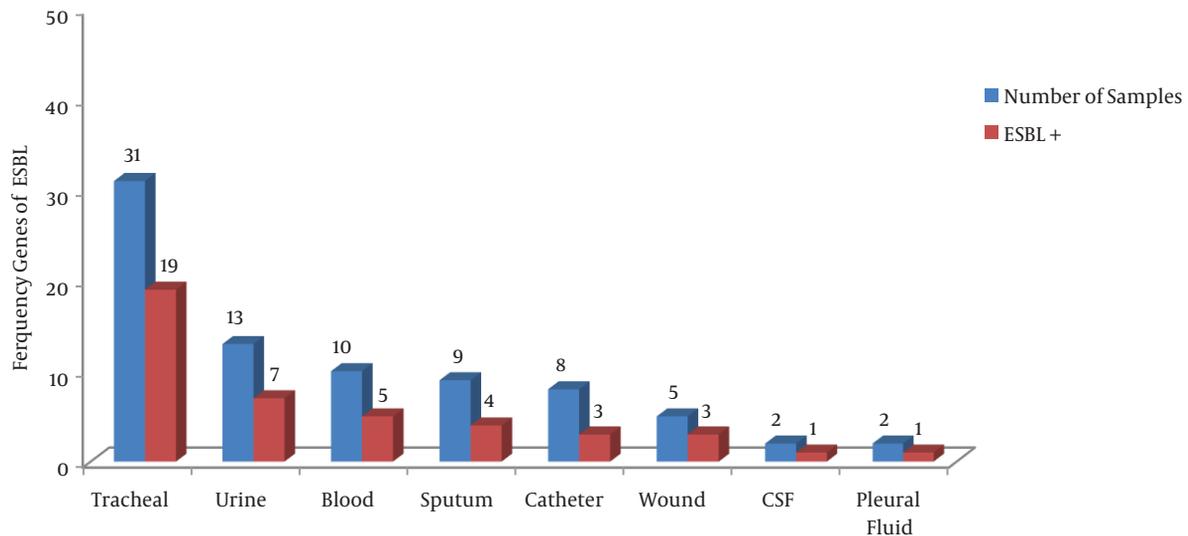
Figure 1. The Frequency of ESBL-Producing Isolates of *A. Baumannii* Isolated from Clinical Specimens Examined

Table 3. The Frequency of ESBL Genes in the MDR Isolates of *A. Baumannii*

Variables	Tracheal	Urine	Blood	Sputum	Catheter	Wound	CSF	Pleural Fluid	Total
MDR	19	9	6	6	5	4	1	0	50
SHV	8	1	4	0	1	1	0	0	15
TEM	6	1	2	0	1	0	0	0	10
CTX-M	0	0	0	0	1	0	0	0	1

more commonly obtained from catheter samples (17). Various factors, including consumption of antibiotics, clonal spread of resistant microorganisms, and drug resistance

mechanisms of different species, result in the dissemination of highly resistant pathogens to commonly used antibiotics. Previous research reported increased resistance

Figure 2. Gel Electrophoresis of PCR Products of ESBL

TEM, 1- Lader (100 bp), 2- Negative control, 3- Positive control (800 bp), 4, 5- Positive sample (800 bp); SHV, 1- Lader (100 bp), 2- Positive control (867 bp), 3- Negative control, 4 - 6- Positive sample (867 bp); CTX-M, 1- Lader (100 bp), 2- Negative control, 3- Positive control (594 bp), 4- Positive sample (594 bp).

of *A. baumannii* to various antibiotics and MDR strains hampered the recovery of patients by lowering the efficacy of many common antibiotics (18). The frequency of *A. baumannii* isolates detected in the present study (62.5%) was in line with that reported by Mirnejad et al., who found that the prevalence of MDR *A. baumannii* isolates ranged from 32.7% to 93% (19).

In the current study, the highest rate of antibiotic resistance was observed against ceftriaxone (100%), amikacin (96.2%), ceftazidime (95%), and cefotaxime (91.2%), and the highest rate of antibiotic susceptibility was observed against polymyxin B (13.8%). Furthermore, 95.4% of the *A. baumannii* isolates were resistant to various cephalosporins. This finding is consistent with the results of other studies, which reported that 60% - 100% of *A. baumannii* isolates were resistant to this class of drugs (19-22). In a study by Kheltabadi et al., only 20% of isolates were resistant to ampicillin/sulbactam (20). In contrast, in the present study, 52.5% of the isolates showed resistance to ampicillin/sulbactam. Due to its ability to inhibit β -lactamases, ampicillin/sulbactam is generally used to treat respiratory and urinary infections. The discord in the findings may be due to differences in the pattern of consumption of antibiotics in this area, as well as increased *Acinetobacter* resistance to ampicillin/sulbactam.

Carbapenems are one of the most commonly used antibiotics to treat bacterial infections. Hujer et al. reported that only 25% and 20% of *A. baumannii* isolates were resis-

tant to the carbapenems meropenem and imipenem, respectively (23). However, recent reports pointed to the increased *A. baumannii* resistance to carbapenems (17, 21, 22), in common with the observations in the present study.

Among various drug resistance-inducing mechanisms, ESBLs play an important role in resistance to commonly used antibiotics, such as penicillin and cephalosporins. Due to the widespread dissemination among nosocomial pathogens via plasmids and integrons, ESBL genes can cause further expansion of drug resistance, including MDR isolates (14, 24). Various studies have reported frequencies of ESBL genes in *A. baumannii* ranging from 25% to 93.45% (25-28). In the present study, among the 80 examined isolates of *A. baumannii*, 53.8% were ESBL producers, which is consistent with the results of previous studies. Among the study genes, *SHV* (41.9%) was the most frequent, followed by *TEM* (25.6%). Similarly, Safari et al. reported that *SHV* (58%) and *TEM* (20%) were the most frequent ESBL genes detected in their study (14). In a study conducted in Iraq, Azhar et al. reported that *SHV* (25%) was the most frequently detected ESBL gene (29).

In line with the findings of the present research, another study also found that the prevalence of *SHV* gene was higher in *A. baumannii* isolates (30). Khalilzadegan et al. reported that *CTX-M* (19.4%) and *TEM* (3.2%) were the most frequently detected ESBL genes (21). On the contrary, in the present study, the most frequent gene was *SHV*. *SHV* gene is originated from *Klebsiella pneumoniae* (31). A high fre-

quency of *K. pneumoniae* bearing SHV gene could likely explain the increased spread and diffusion of SHV gene in bacterial isolates, especially *A. baumannii* in hospital settings in Kermanshah (32). Various factors can explain the discord in reports on the frequencies of ESBL-producing isolates. These include differences in patterns, especially overuse and misuse, of the consumption of antibiotics; differences in the types of antibiotics used; prolonged hospital stays; use of invasive techniques in diagnoses; and differences in diagnostic methods used to identify genes (33).

6. Conclusion

In this study, *A. baumannii* isolates exhibited high levels of resistance against the study antibiotics, and a high percentage of the isolates showed MDR. At present, polymyxin B appears to be the only effective antibiotic in the treatment of infections induced by *A. baumannii*. The frequency of ESBL-encoding genes in *A. baumannii* isolates may be due to overuse and inappropriate use of antibiotics in the region. To combat overuse and prevent further development of drug resistance in Kermanshah, Iran, more attention should be paid to prescribing practices for various antibiotics, including cephalosporins.

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Footnotes

Authors' Contribution: Kamal Ahmadi designed and coordinated the study, participated in most of the experiments, and prepared the manuscript. Mohsen Azizi provided assistance in the design of the study, coordinated and carried out all the experiments, and participated in manuscript preparation. Misagh Etemadimajed and Mahsa Kashef provided assistance with all the experiments. Seyed Hamidreza Mortazavi, Siavash Vaziri, Simin Gheini, and Amirhooshang Alvandi were involved in the statistical analysis, data collection, and drafting the manuscript. All the authors read and approved the content of the manuscript.

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