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Research Article

Determination of Antibiotic Resistance Pattern of Organisms Isolated from Endotracheal Tube Cultures of Patients Admitted to Intensive Care Unit

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ABSTRACT

Background: It is widely accepted that increased prevalence of antibiotic resistance of pathogens grown in the respiratory system in intensive care unit (ICU) patients is a very serious problem causing expansion of mortality. The most important strategy to prevent the occurrence and appropriate solution to control the antibiotic resistance is to thoroughly investigate the pattern of resistance in the studied ward. Therefore, the purpose of this study was to determine the antibiotic resistance pattern of organisms isolated from endotracheal tube secretions of patients admitted to ICU of Khatam-Al-Anbia Hospital at Zahedan in Iran.

Methods: In the present retrospective and descriptive cross-sectional study, the medical records of patients hospitalized during 2013-2018 were included by census method and then selected based on inclusion criteria (n=1387). The required data, including age, gender, type of microorganism isolated from endotracheal tube cultures, antibiotic resistance and sensitivity, duration of intubation and cause of hospitalization, were recorded for each patient. Finally, the data were analyzed by descriptive statistics using SPSS 16 software.

Results: Mean age of patients was 44.66 ± 21.39 years and mean duration of intubation was 17.96 ± 10.99 days. Stroke was the most common cause of hospitalization with a prevalence of 553 patients (49%). The prevalence of positive culture of endotracheal tube secretions was 1128 (81.3%) of which 71.5% were male (n=807) and 28.5% were female (n=321). The cultures of endotracheal tube secretions resulted in 933 (82.7%) gram-negative bacteria, 191 (16.9%) gram-positive bacteria and 4 (0.4%) mixed isolates. The most prevalent gram-negative bacterium was Acinetobacter baumannii (37.2%) with the highest and lowest antibiotic resistance to Meropenem (95.1% resistance) and colistin (99.5% sensitivity), respectively. In addition, the most prevalent gram-positive bacterium was Staphylococcus epidermidis (50.3%) with the highest and lowest antibiotic resistance to Meropenem (85.7% resistance) and Vancomycin (92.2% sensitivity).

Conclusion: The findings of the present study illustrate that there was widespread bacterial resistance to respiratory tract infections in our ICU patients. Due to the high sensitivity of gram-negative bacteria to colistin, the use of antibiotic combination with colistin in the control of pulmonary infections caused by these organisms can be a good choice. In addition, in the case of gram-positive bacteria, the highest

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sensitivity was to vancomycin; therefore, it can be the selective antibiotic to control infections caused by these bacteria.

Among different complications, ventilator-associated pneumonia (VAP) is the most common nosocomial infections (NIs) that occurs in the intensive care unit (ICU) and affects about 20% of ICU patients and 30% of patients with mechanical ventilation (1). Numerous references have described the incidence of lung infection within 48 hours after admission and artificial airway placement as VAP (2, 3). On the other hand, others also consider the incidence of lung infection within the first 24 hours as VAP (4). This sensitivity to distinguish this pneumonia from other lung infections is because hospital pathogens such as Pseudomonas aeruginosa, Acinetobacter baumannii or methicillin-resistant Staphylococcus aureus are resistant to some or all of the antibiotics, and the resulting infections lead to increased mortality rate in 13-25.5% of patients (5, 6). Prolonged length of hospital stay increases the hospital costs (7). It should be noted that 50% of antibiotics are prescribed in the ICU for VAP control (8). It is projected that 10 million people will die each year by 2050 due to antibiotic resistance in the absence of any universal approach taken to control this issue (9, 10). In a study in one of the ICUs in Iran, Enterobacter and P. aeruginosa were identified as the most common organisms causing VAP, and acknowledged that gram-negative bacilli were most sensitive to imipenem and gram-negative organisms were resistant to all antibiotics in eight cases (11). In another study, the most common organisms causing VAP were A. baumannii, P. aeruginosa, Klebsiella pneumoniae, Escherichia coli, Stenotrophomonas maltophilia, and Serratia marcescens. They reported that almost all organisms had multi-antibiotic resistance; the highest prevalence (54%) was related to acinetobacter, which was resistant to ciprofloxacin and piperacillin-tazobactam but ampicillin-tazobactam had a good effect (12). In another hospital in India, tracheal secretion was positive in most patients and several species of bacteria were grown simultaneously in some patients, but the most common bacterium was K. pneumoniae. The highest sensitivity was observed between the combination drugs of Cefeperazone-salbactam and Pipperacillin-tazobactam, with over 60% sensitivity among gram-negative bacteria and 100% sensitivity to vancomycin and linezolid among the gram-positive bacteria (13). In another study in Pakistan, the main bacteria causing VAP were A. baumannii (39.8%) and K. pneumonia (12.3%) and methicillin-resistant S. aureus (11.5%), and polyamycin B was the most appropriate drug for the treatment of patients infected with acinetobacter, which had a sensitivity of 64%, while vancomycin and linosolide had 100% sensitivity for methicillin-resistant S. aureus (14). The results of numerous studies show that the antibiotic resistance pattern varies in different geographical areas and there is some degree of resistance to new generation antibiotics, which can be an alarm and persuade physicians to monitor periodically the antibiotic resistance patterns and use these models for experimental and specific treatment of infections (15, 16). Therefore, it is necessary for each region to monitor its resistance and sensitivity patterns not cross-sectionally but dynamically and sustainably so that the results can be an appropriate guide for the proper administration of antibiotics in that region, and this action in particular in the ICU is of greater importance (11, 17). The aim of this study was to determine the antibiotic resistance pattern of organisms isolated from endotracheal tube cultures of patients admitted to ICU of Khatam-Al-Anbia Hospital at Zahedan in Iran.

Methods

The present retrospective and descriptive cross-sectional study was conducted after obtaining approval from the Ethics Committee (IR.ZAUMS.REC.1397.179) and the necessary authorities from the Deputy of Research and Technology at the Zahedan University of Medical Sciences, and their submission to affiliated hospitals. According to previous studies, the sample size was estimated to be 1100 (18). To ensure the adequacy of the required sample size, all medical records of patients admitted during the years 2013-2018 were enrolled in the study, and were selected to analyze data based on inclusion criteria. At last, 1387 samples were selected for final analysis.

The inclusion criteria were over 18 years of age, at least 2 days of intubation and 5-day survival after intubation.

Patient information, including gender, age, positive or negative culture of tracheal secretions, type of cultured microorganism, sensitivity and resistance to tested antibiotics, duration of intubation and underlying disease, was recorded for each patient. At the end of the study and after completing the sample size, data were analyzed by SPSS 25 software using descriptive statistics including mean, standard deviation and frequency.

Results

Of the 1387 patients studied, 1128 (81.3%) had positive tracheal secretion culture. Of the 1128 patients with positive cultures of tracheal secretions, 71.5% (807) were male and 28.5% (321) were female. The mean age of the

patients was 44.66 ± 21.39 years. The youngest was 18 years and the oldest was 108 years (Table 1). In the patients with positive bacterial culture, 933 (82.7%) were gram-negative, 191 (16.9%) were gram-positive and 4 (0.4%) were mixed bacteria. The isolated 933 gram-negative bacteria included A. baumannii (n=347, 37.2%), P. aeruginosa (n=268, 28.7%), Enterobacter aerogenes (n=91, 9.8%), K. pneumonia (n=80, 8.6%), E. coli (n=60, 6.4%), Klebsiella oxytoca (n=42, 4.5%), Citrobacter (n=40, 4.3%), Proteus (n=3, 0.3%), Providencia rettgeri (n=1, 0.1%) and Stenotrophomonas maltophilia (n=1, 0.1%). In addition, 191 isolated gram-positive bacteria included 96 (50.3%) Staphylococcus epidermidis, 90 (47.1%) S. aureus, 3 (1.6%) Streptococcus, 1 (0.5%) Enterococcus and 1 (0.5%) Arcanobacterium haemolyticum (Table 2). Of the gram-negative bacteria, A. baumannii, P. aeruginosa, E. aerogenes, K. oxytoca and Citrobacter had the highest and lowest resistance to meropenem and colistin (with sensitivity of 99.5%, 91.7%, 89.5%, 100% and 93.3%), respectively. K. pneumonia had the highest and lowest resistance to the gentamicin and colistin (with sensitivity of 86.7%).

E. coli had the highest resistance to gentamicin and ciprofloxacin and the lowest resistance to colistin (sensitivity of 92.3%). Proteus had the highest resistance to meropenem and the lowest to ciprofloxacin and imipenem (sensitivity of 100%). P. rettgeri showed no resistance to the tested antibiotics and had sensitivity to amikacin, gentamicin, meropenem and colistin (100%). S. maltophilia had the highest and the lowest resistance to colistin and to gentamicin and meropenem (sensitivity of 100%), respectively. Of the gram-positive bacteria, S. epidermidis had the highest and the lowest resistance to meropenem and vancomycin (sensitivity of 92.2%), respectively. S. aureus had the highest and the lowest resistance to cephalothin and vancomycin (sensitivity of 89.6%), respectively. Streptococcus showed no resistance to the tested antibiotics and had the sensitivity to vancomycin and clindamycin (66.7% and 100%, respectively). Enterococcus had no resistance to vancomycin and was completely sensitive (100%). Mixed growth in the medium showed the highest resistance to gentamicin, cephalothin and clindamycin and the lowest resistance to imipenem (sensitivity of 100%) (Tables 3 and 4). The mean duration of intubation in patients with positive culture was 17.96 ± 10.99 days. The shortest and longest duration of intubation were 2 and 51 days, respectively. Concerning the causes of hospitalization, 1128 patients with positive culture included 553 (49%) stroke, 269 (23.8%) head trauma, 124 (11%) cardiopulmonary arrest, 91 (8.1%) burn, 57 (5.1%) diabetic ketoacidosis, 23 (2%) sepsis and 11 (1%) others.

Table 1– Demographic information of patients

Variables	N (%)
Gender	
Male	807 (71.5)
Female	321 (28.5)
Age	
Mean ± SD	44.66 ± 21.39
Duration of ETT	
Mean ± SD	17.96 ± 10.99
Causes of hospitalization	
Stroke	553 (49)
Head trauma	269 (23.8)
Cardio-respiratory arrest	124 (11)
Burn	91 (8.1)
Diabetic ketoacidosis	57 (5.1)
Sepsis	23 (2)
Others	11 (1)

Table 2- Results from the culture medium

Variables	N(%)
ETT swab cultures	
Positive	1128 (81.3)
Negative	259 (18.7)
Isolated pathogens	
Gram negative	933 (82.7)
Acinetobacter baumannii	347 (37.2)
Pseudomonas aeruginosa	268 (28.7)
Enterobacter aerogenes	91 (9.8)
Klebsiella Pneumonia	80 (8.6)
Escherichia coli	60 (6.4)
Klebsiella oxytoca	42 (4.5)
Citrobacter	40 (4.3)
Proteus	3 (0.3)
Providencia Rettgeri	1 (0.1)
Stenotrophomonas maltophilia	1 (0.1)
Gram positive	191 (16.9)
Staphylococcus Epidermidis	96 (50.3)
Staphylococcus Aureus	90 (47.1)
Streptococcus	3 (1.6)

Table3- Results of antibiogram test for gram-negative bacteria

Bacteria	N (%)	Antibiotic Amikacin				Gentamicin				Meropenem			
		U	S	I	R	U	S	I	R	U	S	I	R
Acinetobacter baumannii	367 (37.2)	71 (20.5)	23 (8.3)	22 (8)	231 (83.7)	214 (61.7)	16 (12)	13 (9.8)	104 (78.2)	183 (52.7)	6 (3.7)	2 (1.2)	156 (95.1)
Pseudomonas aeruginosa	268 (28.7)	111 (41.1)	54 (34.4)	22 (14)	81 (51.6)	178 (66.4)	31 (34.4)	15 (16.7)	44 (48.9)	140 (52.2)	39 (30.5)	13 (10.2)	76 (59.4)
Enterobacter aerogenes	91 (9.8)	36 (39.6)	13 (23.6)	10 (18.2)	32 (58.2)	54 (59.3)	11 (29.7)	8 (21.6)	18 (48.6)	44 (48.4)	14 (29.8)	3 (6.4)	30 (63.8)
Klebsiella Pneumonia	80 (8.6)	25 (31.3)	20 (36.4)	11 (20)	24 (43.6)	56 (70)	10 (41.7)	5 (20.8)	9 (37.5)	50 (62.5)	20 (66.7)	1 (3.3)	9 (30)
Escherichia coli	60 (6.4)	24 (40)	25 (69.4)	8 (22.2)	3 (8.3)	29 (48.3)	16 (51.6)	6 (19.4)	9 (29)	34 (56.7)	19 (73.1)	0 (0)	7 (26.9)
Klebsiella oxytoca	42 (4.5)	8 (19)	3 (8.8)	16 (47.1)	15 (44.1)	30 (71.4)	5 (41.7)	1 (8.3)	6 (50)	33 (78.6)	3 (33.3)	1 (11.1)	5 (55.6)
Citrobacter	40 (4.3)	25 (62.5)	4 (26.7)	0 (0)	11 (73.3)	11 (27.5)	9 (31)	1 (3.4)	19 (65.5)	19 (47.5)	1 (4.8)	0 (0)	20 (95.2)
Proteus	3 (0.3)	1 (33.3)	0 (0)	1 (50)	1 (50)	3 (100)	0 (0)	0 (0)	0 (0)	2 (66.7)	0 (0)	0 (0)	1 (100)
Providencia Rettgeri	1 (0.1)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
Stenotrophomonas maltophilia	1 (0.1)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)

Bacteria	N (%)	Antibiotic Ciprofloxacin				Imipenem				Colistin			
		U	S	I	R	U	S	I	R	U	S	I	R
Acinetobacter baumannii	367 (37.2)	184 (53)	9 (5.5)	13 (8)	141 (86.5)	201 (57.9)	14 (9.6)	28 (19.2)	104 (71.2)	149 (42.9)	197 (99.5)	1 (0.5)	0 (0)
Pseudomonas aeruginosa	268 (28.7)	102 (38.1)	57 (34.3)	17 (10.2)	92 (55.4)	95 (35.4)	71 (41)	20 (11.6)	82 (47.4)	123 (45.9)	133 (91.7)	4 (2.8)	8 (5.5)
Enterobacter aerogenes	91 (9.8)	53 (58.2)	11 (28.9)	11 (28.9)	16 (42.1)	47 (51.6)	15 (34.1)	9 (20.5)	20 (45.5)	72 (79.1)	17 (89.5)	0 (0)	2 (10.5)
Klebsiella Pneumonia	80 (8.6)	22 (27.5)	31 (53.4)	8 (13.8)	19 (32.8)	29 (36.3)	36 (70.6)	0 (0)	15 (29.4)	50 (62.5)	26 (86.7)	2 (6.7)	2 (6.7)
Escherichia coli	60 (6.4)	29 (43.8)	17 (54.8)	5 (16.1)	9 (29)	27 (45)	21 (63.6)	3 (9.1)	9 (27.3)	34 (56.7)	24 (92.3)	1 (3.8)	1 (3.8)
Klebsiella oxytoca	42 (4.5)	11 (26.2)	17 (54.8)	4 (12.9)	10 (32.3)	16 (38.1)	13 (50)	4 (15.4)	9 (34.6)	34 (81)	8 (100)	0 (0)	0 (0)
Citrobacter	40 (4.3)	27 (67.5)	1 (7.7)	0 (0)	12 (92.3)	21 (52.5)	1 (5.3)	0 (0)	18 (94.7)	10 (25)	28 (93.3)	1 (3.3)	1 (3.3)
Proteus	3 (0.3)	1 (33.3)	2 (100)	0 (0)	0 (0)	1 (33.3)	2 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)
Providencia Rettgeri	1 (0.1)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
Stenotrophomonas maltophilia	1 (0.1)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)

Table abbreviation
N (%): Number of positive cultures (Percent)
U: Untested
S: Sensitive
I: Intermediate
R: Resistance

Table 4- Results of antibiogram test for gram-positive bacteria

Bacteria	N (%)	Antibiotic Clindamycin				Meropenem				Imipenem			
		U	S	I	R	U	S	I	R	U	S	I	R
Staphylococcus epidermidis	96 (50.3)	62 (64.6)	14 (41.2)	0 (0)	20 (58.8)	82 (85.4)	1 (7.1)	1 (7.1)	12 (85.7)	79 (82.3)	7 (41.2)	2 (11.8)	8 (47.1)
Staphylococcus aureus	90 (47.1)	74 (82.2)	3 (18.8)	1 (6.3)	12 (75)	76 (84.4)	6 (42.9)	3 (21.4)	5 (35.7)	75 (83.3)	7 (46.7)	6 (40)	2 (13.3)
Streptococcus	3 (1.6)	1 (33.3)	2 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)
Enterococcus	1 (0.5)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Bacteria	**N (%)**	**Antibiotic Cephalothin**				**Ceftriaxone**				**Vancomycin**			
		U	S	I	R	U	S	I	R	U	S	I	R
Staphylococcus epidermidis	96 (50.3)	76 (79.2)	6 (30)	1 (5)	13 (65)	78 (81.3)	1 (5.6)	11 (61.1)	6 (33.3)	19 (19.8)	71 (92.2)	2 (2.6)	4 (5.2)
Staphylococcus aureus	90 (47.1)	50 (55.6)	3 (7.5)	1 (2.5)	36 (90)	66 (73.3)	3 (12.5)	7 (29.2)	14 (58.3)	14 (14.4)	69 (89.6)	7 (9.1)	1 (1.3)
Streptococcus	3 (1.6)	3 (100)	0 (0)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.7)	1 (33.3)	0 (0)
Enterococcus	1 (0.5)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)

Table abbreviation
N (%): Number of positive cultures (Percent)
U: Untested
S: Sensitive
I: Intermediate
R: Resistance

Discussion

The results of the present study showed that VAP is highly prevalent in ICU patients, and most of the organisms causing pneumonia are gram-negative bacteria, which have very high antibiotic resistance and are resistant to almost most commonly used antibiotics in the ICU. Among these, Acinetobacter, which is highly prevalent, has multidrug resistance, and among the antibiotics studied, only colistin was able to somewhat control the growth of the bacterium and the prevalence of pneumonia caused by this bacterium was high, similar to other studies in the ICUs (5, 6). Consistent with the results obtained in the present study, Ahsan et al. (2016) also reported that the most prevalent microorganisms isolated from the culture of tracheal secretions were gram-negative bacteria (76.13%), followed by fungal pneumonia (17.04%) and finally gram-positive bacteria (6.81%). The most common organisms grown were Acinetobacter, Klebsiella, Candida and Pseudomonas, respectively. Among gram-negative organisms, Acinetobacter, Klebsiella and Pseudomonas were highly resistant to third-generation cephalosporins and fluoroquinolones (> 80%). Resistance to aminoglycosides (> 68%) and imipenem (> 60%) was also higher, whereas Pseudomonas was less resistant to piperacillin-tazobactam compared to Klebsiella and Acinetobacter. All gram-negative organisms with the exception of Proteus were 100% sensitive to colistin. Gram-positive ones were 100% sensitive to netilmycin and vancomycin with variable resistance patterns to other antibiotics (19). A few points are important in the difference between the two studies. One is that the study was performed with a much smaller sample size, but the prevalence of VAP continued to be high. Second, unlike this study, our study was retrospective, with no evidence of in vitro study to detect fungal pneumonia in the data, and even synthetic antibiotics such as piperacillin-tazobactam had not been tested on grown organisms. This is an unsolvable restriction of this study. However, there is complete agreement between the two studies on the type of microorganism grown and that colistin is the best antibiotic for the control of pneumonia caused by gram-negative organisms and vancomycin for gram-positive. Early-onset VAP occurs within the first 2-5 days after

mechanical ventilation, whose leading agents are S. pneumoniae, Hemophilus influenza, methicillin-sensitive S. aureus (MSSA), antibiotic sensitive E. coli, K. pneumoniae, Enterobacter sp, Proteus sp. and Serratia marcescens (20). Late-onset VAP emerges after 4 days of intubation, whose leading agents are MRSA, Acinetobacter sp, P. aeruginosa, extended-spectrum beta-lactamase (ESBL)-producing bacteria (19, 21). In this study, most of the appeared organisms were of the second category, but no general classification was performed to differentiate early-onset from late-onset VAP. However, classifying types of pneumonia is not important with this level of antibiotic resistance of organisms, and targeted administration of antibiotics in proportion to the sensitivity of the organisms is paramount to prevent the spread of bacterial resistance throughout the region and the world. Another study reported a high prevalence of VAP and that the most common organisms involved were Citrobacter and K. pneumoniae, which had a high degree of resistance to carbapenems as common antibiotics used in the ICU, while being sensitive to Polymyxin B (94%) and Tigecycline (96%) (22).

This organism was the fourth and seventh most prevalent in our study, but was highly resistant to carbapenems as described in the study, and was resistant to aminoglycosides; colistin was the best antibiotic to treat pneumonia caused by this organism. There was no study of antibiotics mentioned in the above study. In a study of Singh et al., K. pneumoniae was also the most important causative agent of VAP and was resistant to third-generation cephalosporins and penicillin antibiotics; contrary to the results of both studies, it was sensitive to carbapenems and polymyxin B (23). Malik et al. also reported that the most common organism responsible for VAP was K. pneumoniae, which had only more than 60% sensitivity to combination drugs such as Cefeperazone-salbactam and Pipperacillin-tazobactam. According to the results of the present study, gram-positive organisms were uncommon and had 100% sensitivity to vancomycin and linezolid (13). Jakribettu et al. reported that 44.2% of patients had VAP and the most common organism was Klebsiella and Pseudomonas, respectively, which were resistant to penicillins, fluoroquinolones and cephalosporins but sensitive to piperacillin/tazobactum, cefaperazone/sulbactum and carbapenems (24). Although there are significant differences in the type of common organism and antibiotic resistance and sensitivity between all the studies mentioned, all studies agree that the most problematic organisms causing VAP are gram-negative bacteria and little antibiotic resistance exists between gram-positive bacteria. This difference in prevalence and antibiotic resistance and sensitivity indicates that all hospitals should continuously study the prevalence of VAP-causing agents and identify their antibiotic susceptibility based on available medications so that they can be used if needed in experimental treatment or after determining the result of culture.

Conclusion

According to the results of the present study, the most common VAP-causing organisms are gram-negative bacteria that have high antibiotic resistance and are resistant to most antibiotics commonly used in intensive care units, and should be targeted purposefully with appropriate antibiotics.

Study limitations

Some of the limitations of the present study were retrospective design, lack of evaluating the sensitivity of isolated bacteria to the different antibiotics recommended in the new literature, and failure to evaluate rates of fungal infections and their controlling drugs.

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Authors' contributions

Masoum Khoshfetrat: participation in implementation of research and supervision over data collection and revising this paper

Morteza Sedaghat Kia: participation in implementation of research and data collection

Mohamad Behnampour: participation in implementation of research and data collection

Aliakbar Keykha: Compilation of the paper and Editing this paper carefully

Alireza Ansari Moghadam: Analyzing the data

All authors reviewed, commented and approved the final version.

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