

# Spatio-Temporal Variability of Resistance Phenotypes of *Escherichia coli* Strains Isolated from Drinking Water in the Village of M'pody (Côte d'Ivoire)

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**Abstract:** In M'pody village from Anyama district, a diarrhea epidemic was detected in January 2020. According to the population involved, these diarrhea cases could be linked to consumption of water from rural hydraulic water supply for almost 3 years. Access to safe drinking water is a prerequisite for good health. Poor drinking water quality is responsible for almost 90% of diarrhoeal diseases and 40% of deaths in developing countries. In addition, in recent years, several studies in both Europe and North America have indicated the presence of antibiotic multidrug-resistant (MDR), *Enterobacteriaceae* (including *Escherichia coli*) or genes coding for antibiotic resistance in various aquatic environments. The present work was carried out with the objective of assessing the bacterial contamination of well and borehole water in the locality of M'pody and determining the level of antibiotic resistance of *Escherichia coli* strains isolated from these waters as well as their resistance phenotypes. Samples of well and borehole water were collected and analyzed using membrane filter method and antibiotic susceptibility of *Escherichia coli* strains isolated from these waters was tested using agar diffusion technique in respect with the recommendations of the Antibiogram Committee of the French Microbiology Society. Microbiological analysis of water samples showed that water was contaminated by *E. coli*, well water being more polluted than the water from the borehole. High resistance was observed against amoxicillin (43.8 to 82.1%), amoxicillin-clavulanic acid (37.5% to 52.6%) and ticarcillin (37.5% and 66.1%). High levels of resistance were also observed against cefalotin (18.8% to 53.6%) and cefuroxime (9.4% to 48.2%). No resistance was observed with 3<sup>rd</sup> generation of cephalosporins. Several resistance phenotypes were observed, TRI phenotype dominating followed by PHN phenotype, PBN phenotype and then CBN phenotype. This study revealed existence of resistant strains in the groundwater of M'pody which would justify implementation of a surveillance of bacterial resistance to antibiotics to limit dissemination and transmission to humans.

**Keywords:** Well and Borehole Water, *E. coli*, Bacterial Resistance, Resistance Phenotypes

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## 1. Introduction

Water is a precious and essential natural resource with many

uses. Access to safe drinking water is a prerequisite for good health. According to the WHO estimates, population dependent on unimproved water sources is 884 million, the majority being

in sub-Saharan Africa, where rate of access to safe water, sanitation and hygiene is the lowest in the world [20]. Low availability of drinking water in urban, peri-urban and rural areas forces people to rely on wells and boreholes. If these structures have the advantage of solving problem of water availability, the quality of this commodity is often not guaranteed. According to the WHO [19], two billion of people (more than 50% living in Africa and Asia) consume water contaminated with faecal matter, causing more than 500,000 deaths per year. Poor quality of drinking water is responsible of about 90% of diarrhoeal diseases and 40% of deaths in developing countries [1, 21]. These waterborne diseases are mostly caused by pathogenic bacteria of intestinal origin. Detection of bacteria such as total coliforms, thermo-tolerant coliforms including *Escherichia coli* in water is an evidence of faecal contamination. *E. coli* is the only member of the coliform group found exclusively in the intestines of mammals, including humans, and its detection in water should be considered as reflecting the possible presence of pathogenic microorganisms of faecal or enteric origin [10]. In recent years, several studies in Europe and North America have indicated the presence of multidrug-resistant (MDR) Enterobacteriaceae (including *Escherichia coli*) or genes coding for antibiotic resistance in different aquatic environments [8, 11, 15]. In Africa also and especially in Franceville, Gabon, Yala *et al.* enterobacteria resistant to 3<sup>rd</sup> generation of cephalosporins had been found in waters of lakes and rivers [22]. In Ivory Coast, multi-antibiotic resistant bacteria have been reported in wastewater, hospital effluents and surface water by several authors [3, 6, 9]. Among them, Enterobacteriaceae and mainly *E.*

*coli*, producing extended-spectrum  $\beta$ -lactamases (ESBL), occupy an increasing place. The presence of antibiotic-resistant bacteria in drinking water could therefore be an important link in strewing and circulation of resistance genes carried by these bacteria. In January 2020, following an epidemic of diarrhoea in M'pody, a village located at 60 km about from the town of Anyama, investigations were carried out to identify the causes. The drinking water supply system of the village is made up of Improved Village Hydraulics (IVH) and mostly traditional wells. In the framework of this investigation, water samples were collected from all the wells and the only borehole. This work was therefore carried out with aim of evaluating the bacterial contamination of the well and borehole water in the locality of M'pody and to determine the level of resistance to antibiotics of the *Escherichia coli* strains isolated from these waters and the associated resistance phenotypes in order to measure the health risks to which the population of M'pody is exposed.

## 2. Material and Methods

### 2.1. Presentation of the Study Area

M'pody is a locality of Anyama sub-division located in the division of Abidjan in Ivory Coast. This village, with an estimated population of 2,731 inhabitants according to the 2014 census [12], does not benefit from the public water supply network and relies on wells and an improved village hydraulic system (HVA) including a borehole for its drinking water supply (Figure 1).

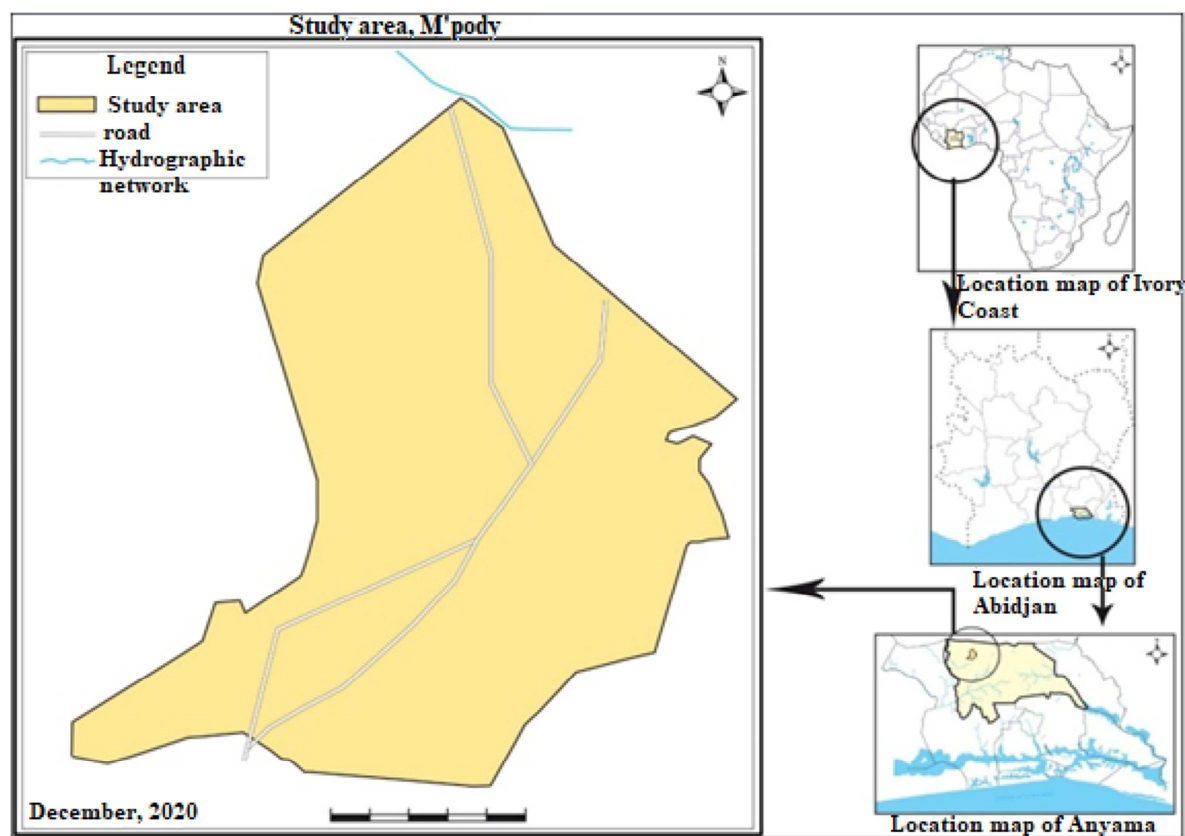


Figure 1. Presentation of the study area.

## 2.2. Material

### 2.2.1. Biological Material

The biological material consisted of water samples taken from the different wells and the HVA.

### 2.2.2. Sample Collection and Transport Equipment

The water samples were collected in sterile glass bottles of 500ML capacity and transported to the laboratory in a cooler equipped with a thermometer and cold accumulators to maintain a temperature range of 4°C to 8°C. Analyses were carried out within four hours of collection.

### 2.2.3. Analytical Material

Research and counting of bacterial agents required use of various equipment, small materials and consumables, including a membrane filtration ramp (Sartorius), filtration membranes of 0.45µm porosity, 55 mm diameter Petri dishes and Rapid'E.coli2 agar (BIO-RAD, France) for culture. Study of sensitivity of bacteria to antibiotics was carried out on Müller Hinton agar (MH) (BIO-RAD, France). Antibiotics tested and their loads (BIO-RAD, France) are recorded in the following table 1.

**Table 1.** List of antibiotics tested.

Families	Sub-families	Antibiotics	Abbreviation	Load (µg)
Beta-lactams	Aminopenicillins	Amoxicillin	AMX	20
		Amoxicillin + IβL	AMC	20 + 10
	Carboxypenicillin	Ticarcillin	ICT	75
	Monobactam	Aztreonam	ATM	30
	Carbapenem	Imipenem	IMP	10
	Cephalosporin 1G	Cephalotin	CF	30
		Cefuroxime	CXM	30
	Cephalosporin 2G	Cefoxitin	FOX	30
	Cephalosporin 3G	Cefotaxime	CTX	30
		Ceftazidime	CAZ	30
Aminosides	Amikacin	Amikacin	AKN	30
		Gentamicin	GEN	30
		Netilmicin	NET	30
Quinolones		Levofloxacin	LEV	5
		Ciprofloxacin	CIP	5
Sulfamethoxazole/trimethoprim		Cotrimoxazole	SXT	1,25+23,75

## 2.3. Methods

### 2.3.1. Type and Period of Study

This prospective and analytical study was carried out on groundwater from the village of M'pody from February to October 2020 and used microbiological techniques, in particular classical bacteriology and biochemical tests to identify isolates.

### 2.3.2. Sampling Method

All wells and single HVA were sampled during four (4) sampling campaigns that took place successively during the months of February, June, August and October 2020 with one sample per site.

### 2.3.3. Research and Counting of Germs

The membrane filtration method was the technique used for microbial research and enumeration. The identification of characteristic *Escherichia coli* colonies was based on rapid biochemical characters (oxidase and catalase) and also on the reduced gallery of Le Minor.

The bacterial strains obtained were stored at -20°C in skimmed milk until antibiotic susceptibility testing was carried out.

### 2.3.4. Study of the Sensitivity of Germs to Antibiotics

This was done by agar diffusion method according to the

recommendations of the CA-SFM 2019. The strains tested were those distributed after plating. For the detection of the ESBL (extended spectrum beta-lactamase) phenotype, disks of 3<sup>rd</sup> generation cephalosporins (cefotaxime, ceftazidime and cefepime) or aztreonam were placed 1.5 cm from the amoxicillin/clavulanic acid combination disk. Presence of a champagne cork image indicated the production of an extended spectrum beta-lactamase. Quality control of the antibiograms was performed with the reference strain: *Escherichia coli* ATCC 25 922.

### 2.3.5. Statistical Analysis

Statistical analysis of the data was carried out using EXCEL software.

## 3. Results

### 3.1. Microbiological Quality of Analysed Waters

Results of the microbiological analyses showed that well waters were all contaminated with *E. coli* and did not comply with evaluation criteria during the 4 sampling campaigns. The borehole water was less contaminated with very low *E. coli* loads detected during the February and June campaigns.

Table 2. *E. coli* loads in the analysed waters.

Campaign month	<i>E. coli</i> load in CFU/100ml		Criteria	Number of satisfactory results	
	Wells (n=72)	Min-Max (value Average)		Well	Drilling
February	1-3400 (413)		0	0	0
June	1-5000 (1116)			0	0
August	1-20000 (1656)			0	1
October	1-2820 (640)			0	1

\*European Union criteria from Council Directive 98/83/EC; n: number of samples analysed; Min: minimum; Max: maximum

### 3.2. Antibiotic Sensitivity of *E. coli* Strains Sensitivity to $\beta$ -lactam

Table 3 shows high resistance to amoxicillin, amoxicillin-I $\beta$ L (clavulanic acid) and ticarcillin with rates ranging from 43.8% to 82.1% for amoxicillin, 37.5% to 52.6% for amoxicillin-clavulanic acid and 37.5% to 66.1% for

ticarcillin respectively. High levels of resistance were also observed against 1<sup>st</sup> (cephalosporins) and 2<sup>nd</sup> generation cephalosporins (cefuroxime) with rates ranging from 18.8% to 53.6% for cefalotin and 9.4% to 48.2% for cefuroxime respectively. No resistance was observed with the 3<sup>rd</sup> generation of cephalosporins. The highest rates of resistance were observed in October.

Table 3. Susceptibility of *E. coli* strains to  $\beta$ -lactams.

Antibiotics	February (n = 32) S (%) R (%)	June (n = 38) S (%) R (%)	August (n = 41) S (%) R (%)	October (n = 56) S (%) R (%)
Amoxicillin	18 (56,2) 14 (43,8)	12 (31,6) 26 (68,4)	15 (36,6) 26 (63,4)	10 (17,9) 46 (82,1)
Amoxicillin + I $\beta$ L	20 (62,5) 12 (37,5)	18 (47,4) 20 (52,6)	21 (51,2) 20 (48,8)	34 (60,7) 22 (39,3)
Ticarcillin	20 (62,5) 12 (37,5)	21 (55,3) 17 (44,7)	22 (53,7) 19 (46,3)	19 (33,9) 37 (66,1)
Aztreonam	32 (100) 0 (0)	38 (100) 0 (0)	41 (100) 0 (0)	56 (100) 0 (0)
Imipenem	32 (100) 0 (0)	38 (100) 0 (0)	41 (100) 0 (0)	56 (100) 0 (0)
Cephalotin	26 (81,2) 6 (18,8)	24 (63,2) 14 (43,8)	30 (73,2) 11 (26,8)	26 (46,4) 30 (53,6)
Cefuroxime	29 (90,6) 3 (9,4)	30 (78,9) 8 (21,1)	33 (80,5) 8 (19,5)	29 (51,8) 27 (48,2)
Cefoxitin	29 (90,6) 3 (9,4)	32 (84,2) 6 (15,8)	33 (89,5) 8 (19,5)	37 (66,1) 19 (33,9)
Cefotaxime	32 (100) 0 (0)	38 (100) 0 (0)	41 (100) 0 (0)	56 (100) 0 (0)
Ceftazidime	32 (100) 0 (0)	38 (100) 0 (0)	41 (100) 0 (0)	56 (100) 0 (0)

I $\beta$ L: beta-lactamase inhibitor

### 3.3. Sensitivity to Other Antibiotics

With regard to other antibiotics, Table 4 shows significant resistance to cotrimoxazole and fluoroquinolones, with rates ranging from 22% to 35.7% for cotrimoxazole and 3% to

37.5% for fluoroquinolones respectively. No resistant strains were observed with aminoglycosides except in October. Highest resistance rates were also observed in October.

Table 4. Sensitivity of *E. coli* strains to other antibiotics.

Antibiotics	February (n = 32) S (%) R (%)	June (n = 38) S (%) R (%)	August (n = 41) S (%) R (%)	October (n = 56) S (%) R (%)
Amikacin	32 (100) 0 (0)	38 (100) 0 (0)	41 (100) 0 (0)	53 (94,6) 3 (5,4)
Gentamicin	32 (100) 0 (0)	38 (100) 0 (0)	41 (100) 0 (0)	53 (94,6) 3 (5,4)
Netilmicin	32 (100) 0 (0)	38 (100) 0 (0)	41 (100) 0 (0)	53 (94,6) 3 (5,4)
Levofloxacin	31 (96,9) 1 (3,1)	31 (81,6) 7 (18,4)	40 (97,6) 1 (2,4)	47 (83,9) 9 (16,1)
Ciprofloxacin	31 (96,9) 1 (3,1)	31 (81,6) 7 (18,4)	40 (97,6) 1 (2,4)	47 (83,9) 9 (16,1)
Cotrimoxazole	20 (62,5) 12 (37,5)	23 (60,5) 15 (39,5)		

### 3.4. Resistance Phenotypes of *E. coli* Strains

Table 5 shows that domination of wild strains was among *E. coli* population tested during the first three campaigns with proportions of 56.3%, 31.6% and 48.8% respectively. Several acquired resistance phenotypes were found, the TRI

phenotype was in majority during the first three campaigns (21.9%, 18.4%, 24.4%) followed by the PHN phenotype (9.4%, 18.4%, 19.5%). During the last campaign, the PHN phenotype was predominant (30.4%) followed by PBN phenotype (25%) and CIP R (10.7%). One ESBL strain was isolated during the last campaign in October.

Table 5. Resistance phenotypes of *E. coli* strains.

Phenotypes	February n (%)	June n (%)	August n (%)	October n (%)
Wild	18 (56,3)	12 (31,6)	20 (48,8)	11 (19,6)
PBN	2 (6,2)	3 (7,9)	0 (0)	14 (25)
PHN	3 (9,4)	7 (18,4)	8 (19,5)	17 (30,4)
TRI	7 (21,9)	7 (18,4)	10 (24,4)	4 (7,1)

Phenotypes	February n (%)	June n (%)	August n (%)	October n (%)
CBN	2 (6,2)	6 (15,8)	3 (7,3)	3 (5,4)
ESBL	0 (0)	0 (0)	0 (0)	1 (1,8)
CIP R	0 (0)	3 (7,9)	0 (0)	6 (10,7)
Total	32 (100)	38 (100)	41 (100)	56 (100)

PBN: Low-level penicillinase; PHN: High-level penicillinase; CBN: Low-level cephalosporinase TRI: Beta-lactamase inhibitor-resistant TEM phenotype; ESBL: Extended-spectrum beta-lactamase CIP R: Ciprofloxacin-resistant

## 4. Discussion

In this study, results of analyses revealed that well waters analysed was all contaminated with *Escherichia coli* strains. In Abengourou, Ivory Coast, Aka et al. [4] found *E. coli* strains in 28% of the well waters analysed. Souny et al [16] reported that 78.1% of well waters samples analysed in Lomé, Togo was contaminated with *E. coli* strains. Presence of *E. coli* indicates a recent faecal contamination, meaning that there would be a probable risk that pathogenic germs are present in these waters. Consumption of these waters exposes the population of this locality to numerous waterborne diseases such as typhoid, dysentery and diarrhoea, as these waters contain high levels of *Escherichia coli* [10]. According to Bricha et al [5], sewage systems, septic tanks and solid waste are the main sources of groundwater pollution in the urban sector and in peri-urban areas. Furthermore, groundwater contamination from wells depends on the permeability of the soil and the depth of the water table [17, 23].

Antibiotic sensitivity tests revealed the presence of *E. coli* strains multi-resistant to the antibiotics tested. Resistance rates varied over time, with the lowest rates observed in February, during the first sampling campaign, compared to the higher rates obtained in October. Among the beta-lactam antibiotics, the lowest rates of resistance to amoxicillin, amoxicillin+clavulanic acid, ticarcillin, cefalotin and cefuroxime were 43.8%, 37.5%, 18.8% and 9.4% respectively. Over the same period, the resistance rates for trimethoprim+sulfamethoxazole and fluoroquinolones were 37.5% and 3.1% respectively. The results of the last sampling campaign (October) showed higher rates of resistance with beta-lactams, with rates for amoxicillin, amoxicillin+clavulanic acid, ticarcillin, cefalotin and cefuroxime being 82.1%, 39.3%, 66.1%, 52.6% and 48.2% respectively.

The rates observed in this study were higher than those reported by Agbessi et al. in [3] in Côte d'Ivoire. Indeed, the study of antibiotic sensitivity of strains isolated from bathing waters showed resistance rates of 50%, 15.4%, 38.5%, 3.8%, 41% and 37.5% respectively to amoxicillin, amoxicillin + clavulanic acid, ticarcillin, cefuroxime, cotrimoxazole and fluoroquinolones. Moreover, 3.1% of the strains isolated from bathing waters were resistant to 3<sup>rd</sup> generation cephalosporins, whereas in the present study, no resistance was observed with 3<sup>rd</sup> generation cephalosporins. In [22], in Franceville, Gabon, the study conducted by Yala et al. on strains of Enterobacteriaceae isolated from lake and river waters revealed resistance rates of 33.3% to the 3<sup>rd</sup> generation of

cephalosporins (ceftriaxone and cefotaxime).

Resistance rates observed in the present study were also higher than those reported by Servais et al. in [14]. Indeed, *E. coli* strains isolated from the Seine River in France showed resistance rates to amoxicillin, cotrimoxazole and ciprofloxacin of 33%, 16% and 2% respectively [14]. Furthermore, these authors found no levofloxacin-resistant isolates, in contrast to the present study where 3.1-18.4% of strains were resistant to levofloxacin. The high level of resistance to trimethoprim/sulfamethoxazole is consistent with several studies suggesting that this antibiotic is a key factor in the emergence of antibiotic resistant *E. coli* strains [13]. According to SCHROEDER et al, [13] about 40% of *E. coli* strains isolated from humans are resistant to trimethoprim/sulfamethoxazole, because this combination of antibiotics is recommended to treat a wide range of human infections.

In addition, 3 to 18.4% of the *E. coli* strains studied were multi-resistant. These strains were resistant to at least three families of antibiotics (beta-lactams, quinolones, sulfamethoxazole-trimethoprim combination). The presence of multi-resistant bacteria in these well waters could be due to the selection pressure exerted by the abusive use of these antibiotics, which are very widely prescribed and most often used as first-line drugs for the outpatient treatment of several bacterial infections. Gram-negative bacilli in general, and enterobacteria (including *E. coli*) in particular, have the capacity and ease of exchanging genetic information with each other or with other bacteria in the environment, which could increase multidrug resistance to antibiotics and promote the spread of this resistance to other germs [18].

Regarding acquired resistance phenotypes, the TRI phenotype was in majority in the first three seasons (21.9%, 18.4%, 24.4%) followed by the PHN phenotype (9.4%, 18.4%, 19.5%). During the last campaign, the PHN phenotype was in majority (30.4%) followed by the PBN phenotype (25%) and CIP R (10.7%). One ESBL strain was isolated during the last campaign in October. In the study by Agbessi et al. [2] the PBN phenotype represented 34.1%, the TRI% phenotype 15.9% while the PHN phenotype was not observed. However, an ESBL-producing strain was identified as in the present study. The presence of ESBL strains in the waters studied, demonstrates the health risk associated with the consumption of M'pody well water. Antibiotic resistance within the *E. coli* species through the production of extended-spectrum beta-lactamase is a public health problem. Indeed, infections due to these multi-resistant bacteria requires treatment with the latest generation of molecules such as carbapenems, the use of which favours the development of additional resistances that can lead to therapeutic impasses [7].

## 5. Conclusion

This study of *E. coli* strains isolated from well and borehole waters in the village of M'pody revealed the presence of strains that were multi-resistant to antibiotics. Among the *E. coli* strains, 43.8% to 82.1% were resistant to amoxicillin, 37.5% to 52.6% to the combination of amoxicillin and clavulanic acid, and 37.5% and 66.1% to ticarcillin. High levels of resistance were also observed against 1<sup>st</sup> (cephalosporins) and 2<sup>nd</sup> generation cephalosporins (cefuroxime) with rates ranging from 18.8% to 53.6% for cefalotin and 9.4% to 48.2% for cefuroxime respectively. No resistance was observed with the 3<sup>rd</sup> generation cephalosporins. The highest resistance rates were observed in the October campaign. Regarding resistance phenotypes, the majority of strains were of wild-type. The acquired resistance phenotypes observed in the first three campaigns were, in decreasing order: the TRI phenotype with respective rates of 21.9%, 18.4%, 24.4% followed by the PHN phenotype with respective rates of 9.4%, 18.4%, 19.5%. During the last campaign, the PHN phenotype was in the majority (30.4%) followed by the PBN phenotype (25%) and CIP R (10.7%). One ESBL strain was also isolated during the last campaign in October. The proportion of multi-resistant strains (3 to 18.4%) indicates the need to monitor these waters in order to implement a strategy to control the dissemination of these strains and limit their transmission to humans.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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