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Distribution and Antibiotic Resistance of *Escherichia coli* on Public Transport Routes in Makassar City: Implications for Resistance Surveillance

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ABSTRACT

The resistance of bacteria to antibiotics is a significant challenge in combating infections. Public transportation can be a hotspot for the spread of antibiotic-resistant bacteria. We surveyed the presence of *Escherichia coli* (*E. coli*) resistant to antibiotics on public transport cars, locally named Pete-Pete, serving four routes in Makassar, Indonesia. Swab samples were collected from door handles, walls, and seats, of 12 public transport vehicles (3 per route across 4 routes). Antibiotic resistance was evaluated using the Kirby-Bauer Disk Diffusion method and the EUCAST standard for resistance determination. We found widespread distribution of antibiotic-resistant *E. coli* at all locations, with variation in resistance patterns between locations. High resistance percentages were found in *E. coli* samples from two routes bordering other districts. Multi-resistant strains of *E. coli* to four types of antibiotics were found in samples from one route bordering the Gowa District. Our research indicates the potential for identifying distribution patterns and detecting levels of antibiotic resistance in pathogens through sampling public transportation.

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INTRODUCTION

Antibiotics, derived from microorganisms, have been crucial in treating bacterial infections, but their overuse has led to widespread resistance (Murray et al., 2022). Based on their mechanism of action, antibiotics can be divided into five categories: (a) antibiotics that inhibit microbial cell metabolism, including cotrimoxazole, sulfonamides, trimethoprim, *para*-aminosalicylic acid (PAS) and sulfones; (b) antibiotics that inhibit microbial cell wall synthesis, including penicillins, cephalosporins, bacitracin, vancomycin, and cycloserine; (c) antibiotics that disrupt the integrity of microbial cell membranes, including polymyxins and polyenes; (d) antibiotics that inhibit microbial cell protein synthesis, including aminoglycosides, macrolides, lincomycin, tetracycline and chloramphenicol; and (e) antibiotics that inhibit microbial cell nucleic acid synthesis, including rifampicin and quinolones (Kapoor et al., 2017).

Resistance of pathogenic bacteria to an antibiotic can occur based on one or more mechanisms, including: microbes synthesizing an enzyme that inactivates or destroys antibiotics, microbes altering their permeability to drugs, microbes developing changes in the target structure for drugs, microbes developing alterations in metabolic pathways directly inhibited by drugs, and microbes developing changes in enzymes that can still perform their metabolic functions but are less affected by drugs than enzymes in susceptible microorganisms (Blair et al., 2015; Davies & Davies, 2010).

Bacterial resistance to antibiotics is a natural phenomenon. However, the continuous use of antibiotics without attention to appropriate dosing accelerates the process of antibiotic resistance. Bacteria develop self-defence mechanisms due to constant antibiotic exposure. Antibiotic resistance constitutes a major threat to public health: antibiotic-resistant bacteria cannot be controlled or killed by antibiotics, allowing them to survive and multiply even in the presence of antibiotic treatment. Most bacteria that cause infections can become resistant to several antibiotics (Laxminarayan et al., 2013).

In developing countries like Indonesia, antibiotic misuse can occur because antibiotics are easily accessible without a doctor's prescription, making them ineffective for treating infectious diseases. Antibiotics are used by the human population and in the agricultural sector to enhance yields (Van Boeckel et al., 2014). In livestock, antibiotics reduce the risk of mortality, restore animals to normal production conditions, and prevent the spread of pathogenic microorganisms. Beyond therapeutic purposes, antibiotics are also used to stimulate growth and accelerate overall animal development (Normaliska et al., 2019; Van Boeckel et al., 2015).

Research has consistently shown that bacteria can spread through touch, contaminating frequently touched objects in various settings. Previous research identified bacterial contamination on hand-touch surfaces in public transport and hospital areas (Otter & French, 2009). Notably, *Escherichia coli* (*E. coli*) has been classified as a critical priority pathogen by the World Health Organisation (WHO, 2017), making it a particularly important target for environmental surveillance. Based on this background, we investigated the spread of antibiotic-resistant *E. coli* in city transportation in Makassar, Indonesia.

Despite global recognition of public transport as a potential vector for antimicrobial resistance, no study has systematically surveyed antibiotic-resistant *E. coli* on public transport vehicles in Makassar City is Indonesia's fourth-largest metropolitan area. Specifically, data are lacking on (1) the prevalence of resistant *E. coli* across different transit routes, (2) variation in resistance patterns between routes connecting urban and peri-urban districts, and (3) the utility of public transport surfaces as sentinel sampling sites for resistance surveillance. This study addresses these gaps by investigating the spread of antibiotic-resistant *E. coli* in city transportation in Makassar, Indonesia.

METHOD

Sampling Locations

Four routes of the Makassar City public transport system, serving the North–South, East–West, and Central areas, were chosen as sampling locations: (a) A. P. Pettarani Street – Hasanuddin University Route (Pettarani – Unhas); (b) Daya Terminal – Central Market Route (Daya – Central Market); (c) Malengkeri Terminal – Cendrawasih Street Route (Malengkeri – Cendrawasih); and (d) Urip Sumohardjo Street – Tamalate Terminal Route (Urip – Tamalate).

These four routes out of six routes were selected because they collectively represent the three primary transit corridors of Makassar City (North-South, East-West, and Central) and include routes that cross administrative boundaries into neighbouring regencies (Maros and Gowa), allowing comparison between intra-city and inter-district routes.

Figure 1. Sampling Locations. Notes: (A–A1) A. P. Pettarani Street – Hasanuddin University Route; (B–B1) Daya Terminal – Central Market Route; (C–C1) Malengkeri Terminal – Cendrawasih Street Route; (D–D1) Urip Sumohardjo – Tamalate Terminal Route

Sampling and Dilution

E. coli was collected from swab samples on the surface of three public transport cars chosen randomly to represent the city transportation serving each route. The surfaces of door handles, door walls, and city transport seats were wiped with a sterile swab over an area of 10 × 10 cm^2. After sampling, the swab was dipped into a screw-cap glass tube containing 0.85% sterile saline. The samples were then transported to the laboratory for *E. coli* isolation and antibiotic-resistance testing.

Cultivation

The saline samples were diluted to 10^{-1}, 10^{-2}, and 10^{-3} with sterile distilled water, and each dilution was filtered on a membrane filter using a vacuum pump. The membrane filters were inoculated on *E. coli* selective chromocult coliform agar (CCA) medium and incubated at 37 °C for 18–24 hours. Colonies were counted in each dilution.

Twenty well-isolated colonies of uniform morphological form were selected from the membrane filters for each sample location. The top of each colony was touched with a sterile toothpick and transferred to a tube containing tryptic soy broth media, then incubated at 37 °C for 18–24 hours. An appropriate dilution of the culture was spread on Mueller-Hinton Agar (MHA) media and incubated at 37 °C for 18–24 hours (CLSI, 2012). This approach ensured well-spread colony growth, allowing the selection of colonies with uniform shape and size for use in the resistance test. Colony growth was facilitated following CLSI cultivation protocols (CLSI, 2012), while resistance breakpoints for disk diffusion were interpreted according to EUCAST guidelines (EUCAST, 2024).

Antibiotic-Resistance Test

We used the Kirby-Bauer Disk Diffusion Method. The following antibiotic disks were applied: amoxicillin-clavulanate (AMC, 20/10 µg), amikacin (AK, 30 µg), chloramphenicol (C, 30 µg), norfloxacin (NOR, 10 µg), and trimethoprim (W, 5 µg). The diameter of the inhibition zone determines the sensitivity or resistance of a bacterium to an antibiotic, visible as a clear area of no bacterial growth around the disk. This inhibition zone depends on the antibiotic type and bacterial strain. We followed the EUCAST protocol to determine resistance strains (EUCAST, 2024). Bacterial isolates resistant to three or more antibiotic classes were classified as multidrug-resistant (MDR), following the internationally accepted standard definition (Magiorakos et al., 2012).

Quality control was performed using *E. coli* ATCC 25922 as a reference strain for disk diffusion. Sterile saline blanks were included as negative controls at each sampling batch. Antibiotics were selected based on their clinical relevance in Indonesia (amoxicillin- clavulanate and trimethoprim are commonly prescribed empirically; amikacin represents a reserve option), their inclusion in WHO's AwaRe classification, and their representation of different mechanism classes (cell wall synthesis, protein synthesis, nucleic acid synthesis, and folate synthesis inhibitors).

Data Analysis

We calculated the resistance rate (RR), defined as the ratio of resistant strains to total strains at each location. Resistance rates were visualised in histograms using Microsoft Excel (version 2019) to compare resistance rates between sampling sites. Principal Component Analysis (PCA) was also performed using Jamovi (version 2.4.11.0) to determine the distribution pattern of bacterial resistance. While the sample size (12 vehicles, 80 isolates) is comparable to previous environmental surveillance studies on public transport, we acknowledge this as a limitation that should be addressed in future larger-scale studies.

RESULTS

Prevalence of Antibiotic-Resistant E. coli

Escherichia coli (*E. coli*) was detected on various surfaces within public transport vehicles, including door handles, door walls, and seats. Antibiotic-resistant *E. coli* strains were found at all four sampled routes. The Daya – Central Market Route exhibited the highest colony count, totalling 1,105 colonies, comprising 57 *E. coli* colonies and 1,048 other coli (Figure 2).

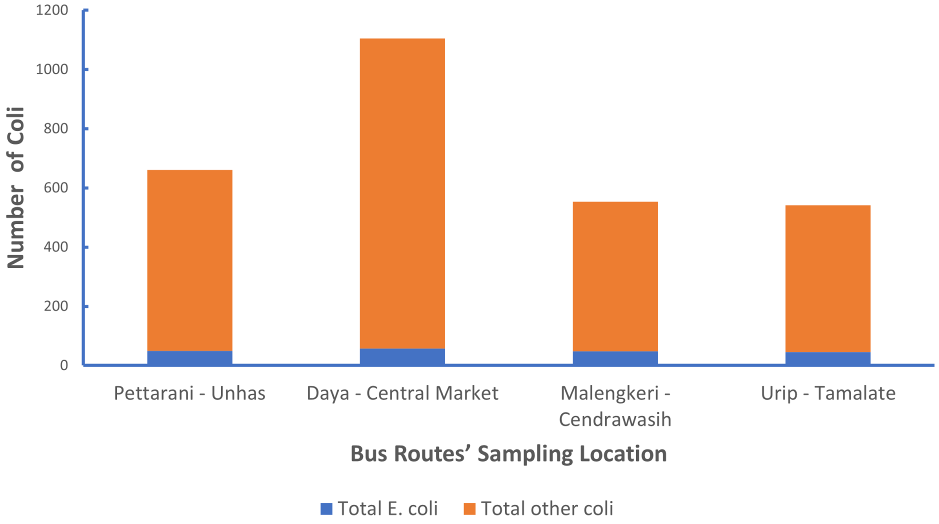

Figure 2. Total E. coli Colonies and Other Coli in Each Location

Variability in Antibiotic Resistance

Resistance rates varied among routes. The highest norfloxacin resistance was found on the Daya – Central Market Route (45%, 95% CI: 28.1-63.0%) and the Malengkeri – Cendrawasih Route (40%, 95% CI: 23.9-58.4%) (Figure 3). *E. coli* from the Malengkeri – Cendrawasih Route showed resistance to four antibiotic classes (amoxicillin-clavulanate, chloramphenicol, norfloxacin, trimethoprim). The Urip – Tamalate Route showed resistance to three classes (chloramphenicol, norfloxacin, trimethoprim). On the Pettarani – Unhas Route, no isolate was resistant to two or more antibiotics (Figure 4). No resistance to amikacin was observed on any route.

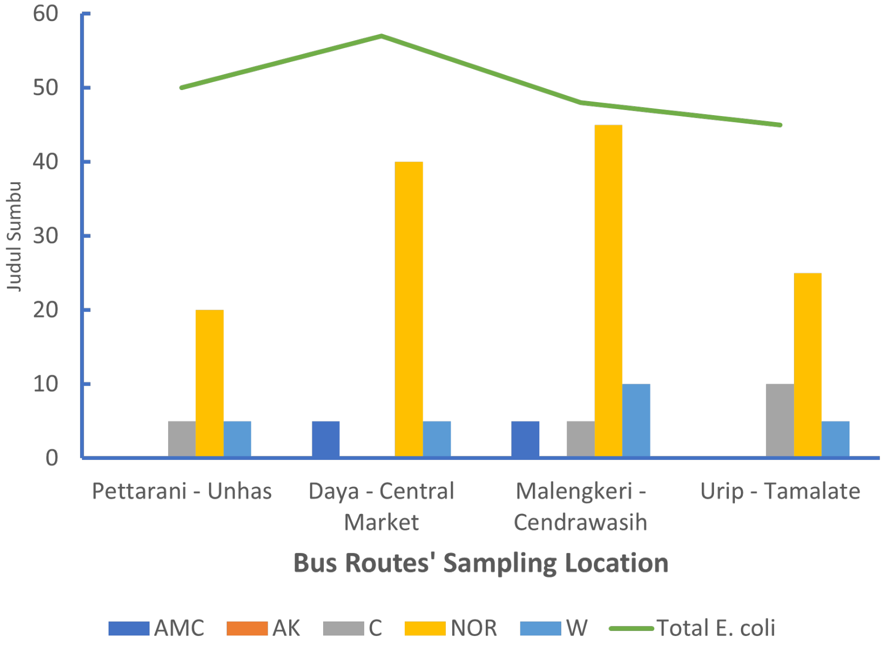

Figure 3. Percent of Colonies Resistant to Antibiotics Following EUCAST Protocol. Notes: AMC = Amoxicillin-clavulanate, AK = Amikacin, C = Chloramphenicol, NOR = Norfloxacin, W = Trimethoprim

The Urip – Tamalate Route and the Malengkeri – Cendrawasih Route both exhibit multi-drug resistance (MDR), with the former resistant to chloramphenicol, norfloxacin, and trimethoprim, and the latter to amoxicillin-clavulanate, chloramphenicol, norfloxacin, and trimethoprim (Magiorakos et al., 2012; Sugianli et al., 2017). Meanwhile, on the Pettarani – Unhas Route, no colonies were resistant to two or more antibiotics (Figure 4).

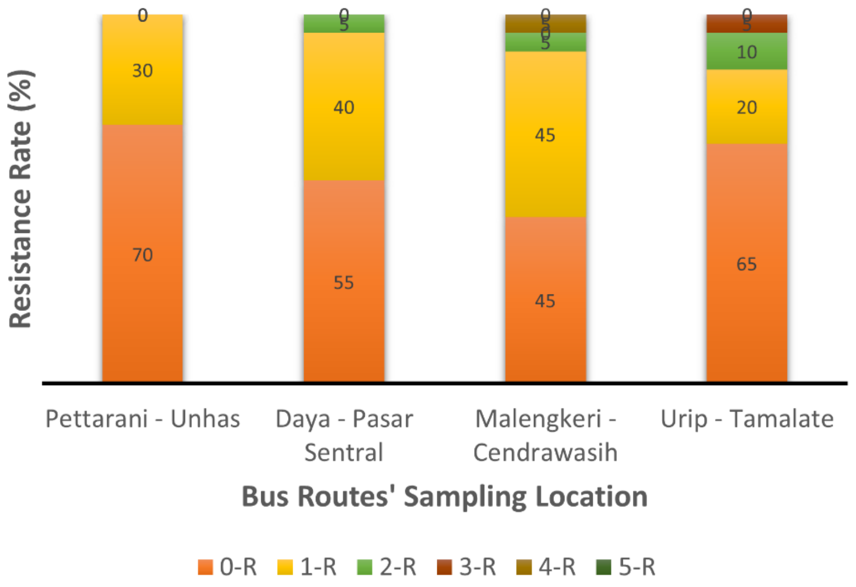

Figure 4. Percent of Colonies Not Resistant (0-R) and Resistant to 1 (1-R) or More (2-R, 3-R, 4-R, 5-R) Antibiotics

Patterns of Resistance Distribution

Our PCA results showed variability in resistance distribution, with two principal components explaining 65.9% of the variation (Figure 5). The Kaiser-Meyer-Olkin measure (0.62) indicated sampling adequacy was acceptable but modest. Given the limited sample size (n = 20 isolates per location, total n = 80), PCA results should be interpreted as exploratory. *E. coli* isolates from the Pettarani – Unhas Route showed a similar resistance pattern to those from the Daya – Central Market Route, while isolates from the Malengkeri – Cendrawasih Route showed a distinct pattern.

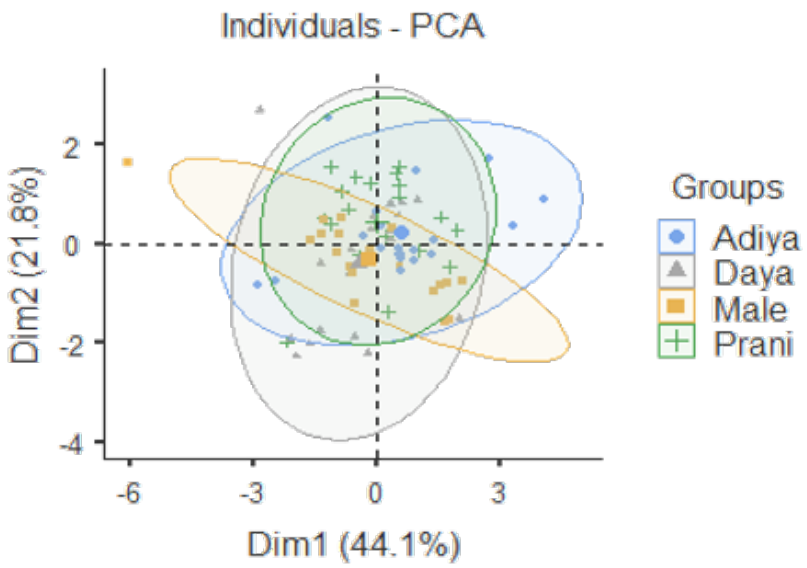

Figure 5. Resistance Distribution Pattern using PCA Labels represent resistance distribution of E. coli isolates from Pete-Pete serving Pettarani–Unhas (Adiya); Daya–Central Market (Daya); Malengkeri–Cendrawasih (Male); Urip–Tamalate (Prani)

DISCUSSION

The detection of antibiotic-resistant *E. coli* on all four sampled public transport routes in Makassar City confirms that public transportation surfaces are reservoirs of resistant bacteria. Disparities in colony counts across routes may reflect differences in route length and passenger use frequency (Cave et al., 2021). The widespread occurrence of *E. coli* in this urban transit network mirrors observations in other public settings at high-contact environmental surfaces, which have highlighted the pervasive nature of antibiotic-resistant *E. coli* in transit systems (Haule et al., 2024; Ly et al., 2024)and public institutions (Tsaku et al., 2017). These findings are also consistent with global patterns of E. coli antibiotic resistance documented in a systematic analysis by Pormohammad et al. (2019).

Our results reveal notable variability in resistance patterns among routes. The highest norfloxacin resistance was observed on routes bordering the regencies of Maros (Daya – Central Market) and Gowa (Malengkeri – Cendrawasih). This finding suggests that norfloxacin may no longer be effective for treating E. coli infections in those areas. Such regional disparities are not unique to Makassar. Other studies have consistently documented significant geographical differences in antibiotic resistance: variations in outpatient antibiotic use across European countries correlate strongly with resistance patterns (Goossens et al., 2005), and similar differences have been reported from clinical isolates across the Netherlands, Belgium, and Germany (Donk et al., 2012). Our findings show analogous differences within a single city, with resistance levels higher on routes that cross administrative boundaries. The correlation between increased antibiotic use and the emergence of resistance has been demonstrated in a systematic meta-analysis (Bell et al., 2014). WHO has emphasised the necessity of continuous surveillance globally (Aarestrup & Woolhouse, 2020; Schnall et al., 2019). These observations highlight the urgent need for targeted interventions to address antibiotic resistance via public transportation systems.

Several local factors may explain the higher resistance observed on routes bordering Gowa and Maros Regencies. First, the inter-regional routes have longer average trip durations compared to intra-city routes, which may allow more time for bacterial transfer and surface colonisation (Nasir et al., 2016; Vargas-Robles et al., 2020). Second, routes connecting to other regencies may experience higher passenger turnover and different passenger demographics (Hsu et al., 2016; Kang et al., 2018), including individuals from areas with intensive livestock farming, known reservoirs of antibiotic-resistant *E. coli* (Peng et al., 2022; Sun et al., 2020). The presence of agricultural zones in Gowa and Maros districts (as noted in the introduction regarding antibiotic use in livestock) suggests potential introduction of resistant strains via commuters (Behrens et al., 2023; Eisenberg et al., 2011; Hickman et al., 2021). These hypotheses warrant target investigation in future studies, including direct measurement of passenger flow, trip duration, and vehicle sanitation practices (Cave et al., 2021).

Multi-drug resistant strain (MDR) strains were identified on two routes: *E. Coli* from the Mallengkeri – Cendrawasih Route showed resistance to four different antibiotic classes (amoxicillin-clavulanate, chloramphenicol, norfloxacin, and trimethoprim), while isolates from the Urip – Tamalate Route were resistant to three classes (chloramphenicol, norfloxacin, and trimethoprim). The presence of MDR *E.coli* on public transport surfaces raises public health concerns, as such strains limit treatment options for infections that may be acquired in community settings. Notably, no resistance to amikacin was found on any route. This is consistent with previous studies conducted in Jakarta and Surabaya hospitals and Sanglah Hospital in Bali, which reported that *E. coli* remains highly sensitive to amikacin in Indonesian clinical settings (Azizah et al., 2025; Fahmi et al., 2022; Junior et al., 2019; Kurniawathi et al., 2021; Lestari et al., 2017; Maulana et al., 2021). These findings pointed out the importance of monitoring local antimicrobial susceptibility data to guide empirical treatment decisions (Bertagnolio et al., 2023; Iskandar et al., 2021; Sartelli et al., 2023).

The distribution of antibiotic resistance in transportation systems is influenced by a complex interplay of factors, including antibiotic use patterns, population dynamics and hygiene conditions (Eisenberg et al., 2011; Olesen et al., 2018; Pruden et al., 2013). These factors likely contribute to the unique resistance patterns observed at different locations. Furthermore, the presence of antibiotic resistance genes in suburban areas has been linked to anthropogenic activities such as waste disposal (Xiang et al., 2018). Broader environmental dimensions of antimicrobial resistance, including the roles of wastewater discharge and anthropogenic inputs, require systematic environmental monitoring (Berendonk et al., 2015; Manaia et al., 2018). Notably, seasonal variations in urban sewage can increase resistance gene concentrations by nearly an order of magnitude, which highlights the critical need for longitudinal monitoring to capture these fluctuations (Su et al., 2017)

The proximity of routes with the highest resistance to agricultural districts suggests potential livestock-to-human transmission pathways, a core concern of the One Health framework. However, more frequent sampling (including seasonal and longitudinal studies) is needed to confirm these observations and to establish causal relationships between specific local factors and resistance patterns. Such studies are essential because, while fecal pollution is a strong predictor of resistance gene abundance, it remains difficult to separate simple dissemination from on-site selection in anthropogenically impacted environments (Karkman et al., 2019).

CONCLUSIONS

Our study demonstrates the widespread distribution of antibiotic-resistant E. coli on public transport surfaces in Makassar City, with resistance rates exceeding 40% for norfloxacin on inter-regional routes. Multi-drug-resistant strains were identified on two routes, and amikacin remained universally effective. Our findings establish public transport surfaces as a practical sentinel surveillance site for environmental antimicrobial resistance monitoring. Limitations include the modest sample size and sampling period, which constrain generalizability. We recommend (1) routine disinfection of high-touch surfaces on public transport, particularly on inter-regional routes; (2) targeted antimicrobial stewardship in Gowa and Maros regencies; and (3) integration of transport surveillance into regional One Health monitoring programs.

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AUTHOR CONTRIBUTION STATEMENT

MAV. wrote the first draft of the manuscript, conducted the sampling, and contributed to data analysis and revision. S. lead the research, designed the methodology, supervised the direction of the manuscript, handled the layout, and revised the draft. N. performed the sampling and carried out the data analysis. Z. M, contributed to data analysis and manuscript revision. All authors reviewed and approved the final version of the manuscript.

AI DISCLOSURE STATEMENT

Grammarly was used only for language proofreading and Perplexity was used only to assist literature searching, while all sources and manuscript content were verified and finalized by the authors.

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