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# Prevalence and Antibiotic Susceptibility of *Proteus mirabilis* Isolated from Clinical Specimens in the Zainoel Abidin General Hospital, Banda Aceh, Indonesia

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**Abstract**

**BACKGROUND:** *Proteus mirabilis* is Gram-negative bacteria from the *Enterobacteriaceae* family causing various infections.

**AIM:** This study aimed to determine the prevalence and distribution of Proteus mirabilis isolated from clinical specimens based on patients' age, gender, type of specimen, and patient wards and their antibiotic sensitivity.

**METHODS:** This study involved isolating, identifying, and testing antibiotic susceptibility to Proteus mirabilis isolates recoverred from clinical specimens of dr. Zainoel Abidin general hospital Banda Aceh during March 2020-March 2022.

**RESULTS:** This study showed that 121 isolates of *Proteus mirabilis* were obtained from clinical specimens during the study with prevalence of almost 2%. *Proteus mirabilis* distribution based on the specimen type was most predominantly found in pus specimens and from patients in the the operating room and post surgery ward accounting for 84.29% and 35.53%. *Proteus mirabilis* was detected most frequently in individuals aged 46-55 years old (30.57 %), whereas it was found more frequently in men (71%) based on gender. The susceptibility of Proteus mirabilis to antibiotics was highest to cefoperazone, piperacillin/tazobactam, and amikacin reaching reaching 97.5%, 97.5, and 96.66%, respectively.

**CONCLUSION:** The distribution of *Proteus mirabilis* isolates predominantly in pus specimens indicating its association with wound infections. Despite some antibiotics remain effective, implementation of the regular surveillance programs along with rational use of antibiotics may prevent the bacterial pathogen to spread particularly within healthcare settings.

## Introduction

Infectious diseases remain the major concerns generating significant implications in both clinical and non-clinical settings. The diseases are causatively associated with the presence of pathogenic microorganisms, including bacteria such as *Proteus mirabilis* that colonizes and invades in the host's body leading to disruption of physiological functions. Commonly characterized as Gram-negative rod-shaped bacteria from the *Enterobacteriaceae* family, *P. mirabilis* is generally known for its urease production as well as ability to develop into elongated swarm cells and distinctive bull's-eye pattern of movement on agar plates [1].

*P. mirabilis* may cause a number of opportunistic and nosocomial infections due to their virulence factors [2]. As pathogenic bacteria, *P. mirabilis* plays important roles as ethiological agents of urinary tract infections, gastrointestinal infections, wound infections, and bloodstream infections. Among those infections, catheter-associated urinary tract infections are most-noted infections for the pathogen [3]. Some virulence factors associated with *P. mirabilis* such as toxins, ureolytic enzymes, flagella, and adhesins facilitate the pathogenic bacteria during infections [4].

In addition to virulence factors, *P. mirabilis* are also capable of forming biofilms. These structures offer the pathogenic bacteria increasing their pathogenicity and enhance resistance to antibiotics [3], [5], [6]. Along with other factors such as irrational use of antibiotics, antibiotic resistance may lead to ineffective treatment, an increase in the risk of patient death and health costs due to the additional hospitalization. Therefore, this study was conducted to determine the prevalence of *P. mirabilis*, distribution of *P. mirabilis* by type of specimen, age, gender, and hospital wards as well antibiotic susceptibility of *P. mirabilis* at the hospital dr. Zainoel Abidin, Banda Aceh, Indonesia.

## Materials and Methods

### *Clinical sample collections*

Blood, urine, sputum, body fluids, pus, and swabs were collected from inpatients and outpatients at the Zainoel Abdin general hospital in Banda Aceh, Indonesia, between March 2020 and March 2022. All clinical samples were evaluated for quality and the types of clinical samples. The research has been approved for ethics from the Ethical Clearance Committee for Health Research, Faculty of Medicine, Universitas Syiah Kuala (No. 400/EA/FK-RSUDZA/2021).

### *Bacterial isolation, observation, and identification*

All clinical samples, except for blood samples which were pre-cultured using BacT/ALERT® 3D (Biomeriux, Lyon, France), were subjected to inoculation on plates containing blood agar (Merck, Germany), MacConkey agar (Merck, Germany) according to the manufacturer's instructions. The plates were then incubated for 24 h at 37°C before they were Gram-stained and macroscopically and microscopically observed for their morphology under microscope with ×1000 magnification.

VITEK® 2 Compact (Biomeriux, Lyon, France), an automated microbiology system utilizing colorimetric reagent cards that are incubated and automatically interpreted to the species level of questioned bacteria, was used for identification and antibiotic susceptibility testing [7]. Briefly, a pure bacterial colony isolated from clinical samples was suspended in NaCl 0.45% solution, which was equivalent to 1.8–2.2 McFarland Standard solution, and then inoculated into suitable cassettes of Gram-negative (GN) for identification and antimicrobial susceptibility testing. Antibiotic susceptibility determination was performed following the guidelines of clinical and laboratory standards using antibiotics as follows: amikacin, ampicillin, amoxicillin, amoxicllin-clavulanate, cefotaxime, cefoxitin, cefoperazone, ceftazidime, ceftriaxone, doxycycline, gentamicin, imipenem, levofloxacin, meropenem, piperacillin/tazobactam, and tobramycin.

### *Statistical analysis*

The distribution of *P. mirabilis* isolates was analyzed descriptively based on the clinical specimens and hospital wards as well as age and gender. Using Microsoft Excel®, all data were tabulated to provide descriptive information in tables or charts. A statistical analysis was performed using Chi-square test or Fisher's exact test when appropriate. All tests performed using XLStat Cloud (Addinsoft, New York, USA) were considered statistically significant at $p \leq 0.05$ on two-tailed.

## Results

### *Bacterial isolation, observation, and identification*

In this study, there was a total of 121 *P. mirabilis* isolates recovered from the clinical specimens. Colonies of *P. mirabilis* growing on the blood agar showed non-hemolytic round-shaped with faded gray, smooth edges, elevated surface, and exhibited circular pattern with spread growth due to the swarming activity (Figure 1). Gram staining results showed that *P. mirabilis* had cells that are Gram-negative red and short rods (Figure 2).

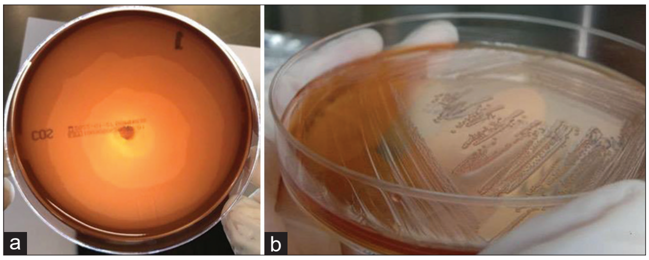


*Figure 1: Growth of Proteus mirabilis showing (a) circle pattern of the swarming phenomenon of colonies on blood Agar media after 24 h and (b) colorless colony growth with round-shaped with faded gray, smooth edges, and elevated surface on MacConkey media after 24 h*

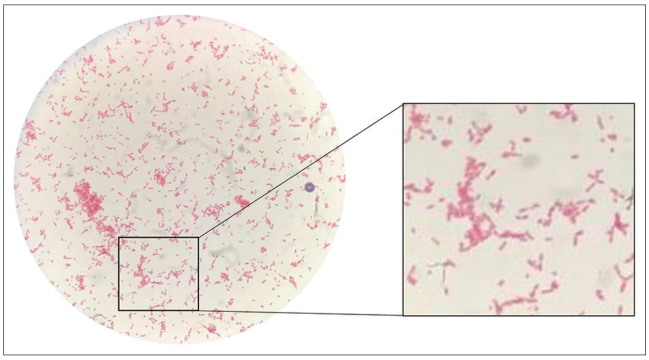

*Figure 2: Cells of Proteus mirabilis Gram staining results showing Gram-negative and short rods observed under a microscope at a magnification of ×1000*

### *Distribution of P. mirabilis isolates based on types of clinical specimens-hospital wards, patients' gender, and age groups*

Distribution of *P. mirabilis* isolates detected based on the types of specimens in a variety of hospital wards of RSUD dr. Zainoel Abidin Banda Aceh is shown in Figure 3. During the period of the present study, there were 6211 clinical samples submitted for testing the occurrence of *P. mirabilis,* which were positive in almost 2% (121 isolates) of those all-clinical samples. Of a total 121 isolates, distribution of *P. mirabilis* isolates was predominantly detected on the pus specimens followed by urine and sputum plus endotracheal tubes accounting for 84.29%, 7.43%, and 6.61%, respectively. The highest number of *P. mirabilis* isolates was obtained from the operating room and post-surgery ward with total 43 isolates (35.53%), followed by the internal medicine room which was counted for 40 isolates (33.05%) out of a total of 121 isolates.

In terms of patients' gender and age, men are more likely to be infected by *P. mirabilis* in this study as the pathogens were more frequently detected in men than women accounting for 86 isolates (70.24%) and 35 isolates (28.92%), respectively (Figure 4). Moreover, *P. mirabilis* isolates were detected predominantly in elderly patients (>45 years) accounting for 71%.

### *Antibiotic susceptibility of P. mirabilis isolates*

Antibiotic sensitivity of *P. mirabilis* isolates collected in this study is shown in Figure 5. *P. mirabilis* isolates were highly sensitive to cefoperazone, piperacillin/tazobactam, and amikacin accounting 97.5%, 97.5%, and 96.7%, respectively. In addition, the pathogenic bacteria were moderately sensitive to cefoxitin (87.5%), meropenem (86.66%), tobramycin (75.8%), ceftazidime (72.5%), amoxicillin-clavulanate (66.67%), ceftriaxone (52.5%), gentamicin (50.83%), and cefotaxime (50%), However, *P. mirabilis* isolates showed lower sensitivity to some antibiotics including

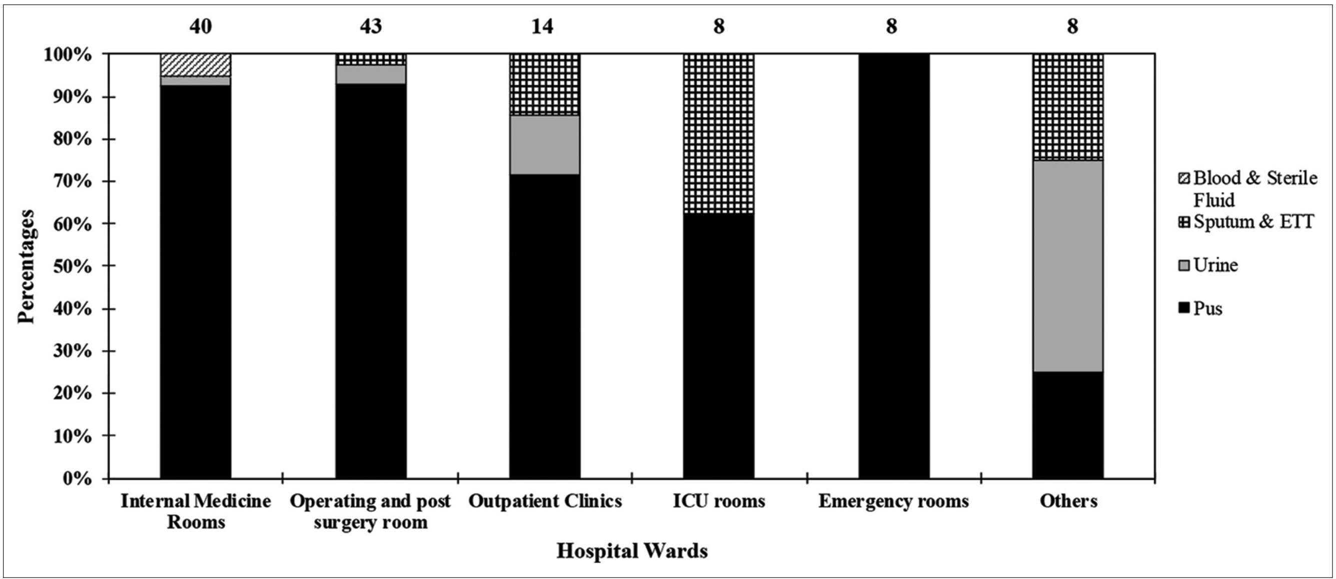


*Figure 3: Frequency of occurrence (%) of Proteus mirabilis isolates (n = 121) distributed based on types of specimens (blood and sterile fluids; sputum and ETT; urine; and pus) collected in a variety of hospital wards (internal medicine rooms; operating and post-surgery rooms; outpatient clinics; ICU rooms; emergency rooms; and others) during a study period of March 2020 – March 2022 at the dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia. Numbers above each column bar are the total number of clinical isolates positive for P. mirabilis. Based on the Chi-square test for independence test, the types of clinical specimens and hospital wards were highly linked (p = 0.0003; $\chi^2$ = 53.62).*

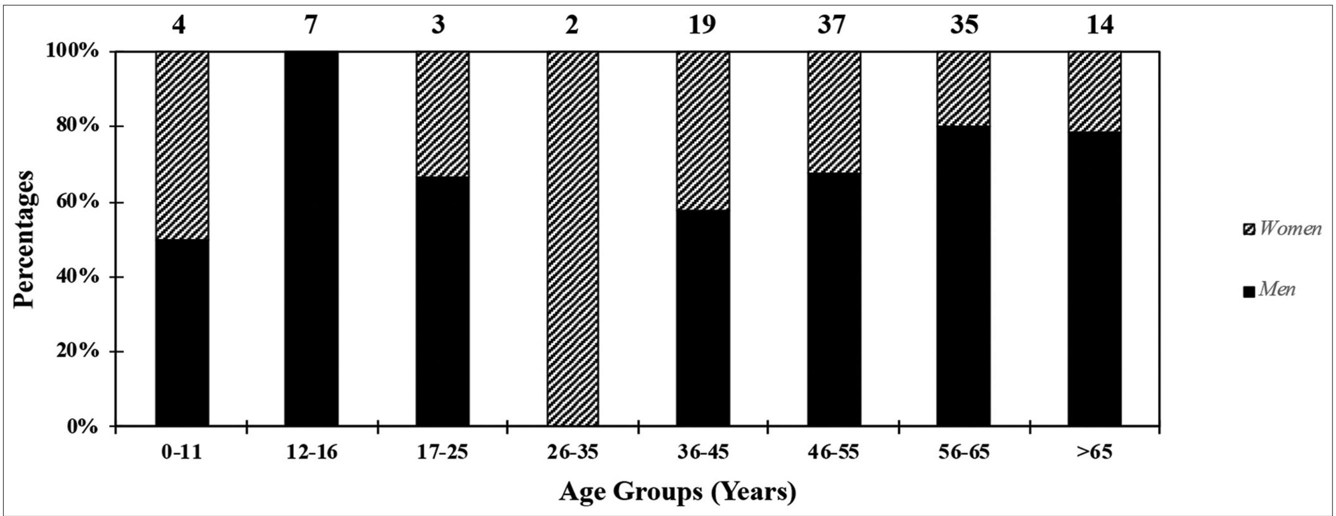


*Figure 4: Frequency of occurrence (%) of Proteus mirabilis isolates (n = 121) based on patients' gender and age groups in the Zainoel Abidin general hospital in Banda Aceh, Indonesia during a period of March 2020 – March 2022. Numbers above each column bar are the total number of P. mirabilis isolates. Based on the Chi-square test for independence test, the patients' gender and age groups were linked (p = 0.049; $\chi^2$ = 12.21).*

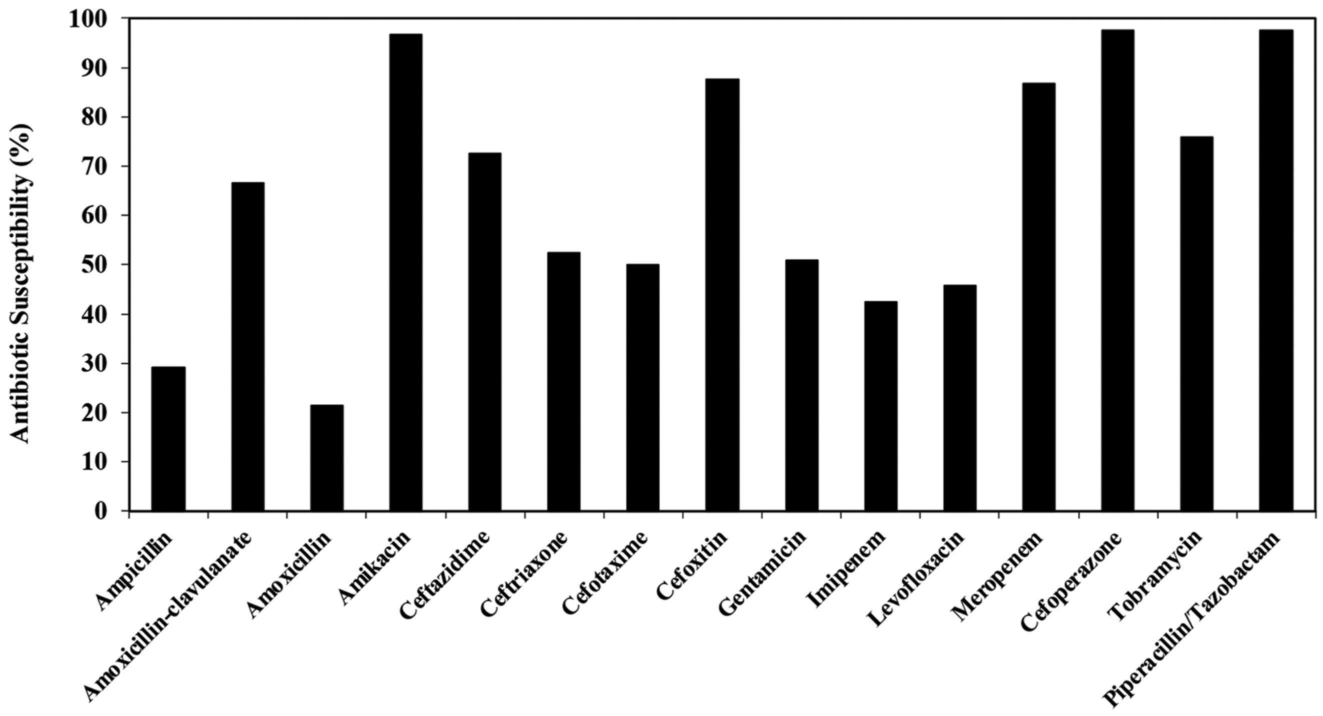


*Figure 5: Antibiotic susceptibility of Proteus mirabilis (n = 121) isolates of clinical specimens collected from patients in the Zainoel Abidin general hospital in Banda Aceh, Indonesia during a period of March 2020 – March 2022*

levofloxacin (45.8%), imipenem (42.5%), ampicillin (29.16%), and amoxicillin (21.55%).

## Discussion

*P. mirabilis* plays an essential function as an etiological agent in various human infections that can be detected by means of isolation, identification, and determination of their antibiotic susceptibility. This study was performed to assess the prevalence, distribution, and antibiotic susceptibility of *P. mirabilis* obtained from a variety of clinical specimens at the dr. Zainoel Abidin's hospital, Banda Aceh, Indonesia. On blood agar, colonies of *P. mirabilis* exhibited circular forms with faded gray, smooth borders, and a raised surface and circular pattern with spread growth. *P. mirabilis* growing on non-selective media such as blood agar are able to form a distinctive pattern due to the swarming, that is, individual or collective cell migration over micrometer-scale distances that are governed by a permissible range of surface conditions [8]. Moreover, the phenomenon of swarming in *P. mirabilis* can occur due to the change of swimmer cells into swarmer cells caused by multicellular differentiation in solid media [1]. These swarming colony distinctive characteristics become major determinants for *P. mirabilis* identification in clinical laboratories. Furthermore, Gram staining results showed that *P. mirabilis* had Gram-negative red and short rods cells. This finding corroborates the previous results signifying the typical GN small straight rod-shaped arranged single or in pairs under the microscope [9].

In the present study, the prevalence of *P. mirabilis* infections was 2% which was higher than previous study accounting for 1% [9]. In addition, *P. mirabilis* isolates were predominantly detected (84.29%) on the pus specimens. These results are in agreement with the previous study [10] demonstrating that *P. mirabilis* commonly found in pus specimens accounting for 58.33% of all clinical specimen positive for *P. mirabilis*. Pus is a sample obtained from a wound infection and can be aspirated pus or swab from the wound as the result of accumulation of dead leukocytes and infectious agents, namely, pyogenic bacteria [11]. Furthermore, this present study also suggests that *P. mirabilis* is prevalently etiological agent for wound infections rather than urinary tract infection [12]. *P. mirabilis is* the most uropathogens after *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. As expected, the present study found that *P. mirabilis* was detected abundantly in urine accounting for 7.43%. Due to its ureolytic biomineralization of the enzyme, *P. mirabilis* become one of main causative agents for complicated urinary tract infections since the enzyme creates urine alkaline and triggers the formation of crystallin biofilm leading to bladder and kidney stone [3]. Other clinical samples, namely, blood and sterile fluid specimens in the present study detected only two isolates (0.09%) from a total of 2132 blood and sterile fluid cultures that were positive for pathogens. The prevalence of *P. mirabilis* infections in blood specimens was considerably low and only accounts for <1% of all cases of blood infections [10]. Most blood infections are caused by *E. coli* and *K. pneumoniae* and *Enterobacter* spp.

The distribution of *P. mirabilis* based on the hospital wards classification showed that this pathogen abundantly found from the operation and the post-surgery room (35.3%), followed by the internal medicine room (33.05%) out of a total 121 *P. mirabilis* detected in this study. This is aligned with aforementioned explanation that *P. mirabilis* was highly related to wound infections as pus samples coming from patients suffering from diabetic ulcers who are treated in the internal medicine room and postoperative wounds treated in the operating-post-surgery room. The previous investigation elucidates that *P. mirabilis* was detected in the wound swab samples collected from diabetic foot ulcer patients in Baghdad [9]. Another study [13] found that the occurrence of *P. mirabilis* infections was almost 14% of all 80 all-positive bacterial infections detected in surgical wound samples.

In the present study, the frequency occurrence of *P. mirabilis* infections in men was more dominant than in women. Similarly, among all of the positive samples, male patients' wounds were more infected with *P. mirabilis* then female patients' wounds [14], [15], [16]. This is in accordance with another study [17] signifying wound infections in men who are higher than in women. Men are generally more involved in strenuous physical activities potentially causing wounds and infections compared to their female counterparts. *P. mirabilis* can infect men and women of all age groups, but the pathogens were more likely to infect those over 45 years of age. This is aligned with previous study demonstrating that *P. mirabilis* infections mostly occurred in elderly patients [18], [19].

Based on antibiotic susceptibility assay, of 121 *P. mirabilis* isolates, 78.45% and 71.84% of those isolates were resistance to amoxicillin and ampicillin, respectively. The bacterial pathogens also showed resistance to imipenem (57.5%), levofloxacin (54.2 8%), cefotaxime (50%), gentamicin (49.17%), and ceftriaxone (47.5%). These findings attenuate previous study highlighting the resistance to major drugs including amoxicillin, carbenicillin, and cefotaxim [19]. However, some antibiotics, namely, cefoperazone, piperacillin/tazobactam, and amikacin, remain effective to treat *P. mirabilis* infections. Other findings also found that aminoglycosides such as gentamicin and amikacin remain effective against *P. mirabilis* infections [10], [12], [14]. In addition, others found similar results, but it showed a significant decrease of the bacterial pathogen against those antibiotics within 10 years [20]. Another study conducted in a hospital in Dhaka Bangladesh demonstrated the emergence of multi drug resistant *P. mirabilis* particularly the biofilm-producing bacterial pathogens that should be taken into consideration [6]. Therefore, the antibiotic resistance surveillance program should be regularly implemented to monitor and evaluate the emergence of the pathogenic bacteria of *P. mirabilis* as well as to prevent their widespread in both communal and clinical settings.

## Conclusion

In general, the prevalence of *P. mirabilis* infections by from March 2020 to March 2022 was almost two per cent. Based on its distribution, *P. mirabilis* was predominantly found in the pus specimens indicating its association with wound infections as it was commonly found in patients treated in the operating and post-surgery room. Moreover, *P. mirabilis* infections were more prevalent in men and in those aged more than 45 years. Cefoperazone, piperacillin/tazobactam, and amikacin remain effective to treat *P. mirabilis* infections. Despite some antibiotics remain effective, implementation of the regular surveillance programs along with rational use of antibiotics may prevent the bacterial pathogen to spread particularly within health-care settings.

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