

# Clinical Utility of Follow-up Urinary Gram-Stain for Pyelonephritis: A Retrospective Observational Study

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## Research Article

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# Abstract

**Background:** The effectiveness of antibiotic therapy for the treatment of pyelonephritis is typically evaluated by the clinical course and results of urine culture. The purpose of this study was to evaluate the usefulness of follow-up urinary gram-stain.

**Methods:** We analyzed the results of hospitalized pyelonephritis patients treated in our department during the last 6.5 years. We investigated whether follow-up urinary gram-stain within 48 hours after initiating antibiotic treatment can help predict resistance to antibiotics at an early stage.

**Results:** 271 patients were enrolled in the study. The mean age was 84.1 years old, and 204 of them (72%) were female. The diagnostic accuracy of using follow-up gram-stain to predict the presence of antibiotic-resistant bacteria was as follows: sensitivity: 41%, specificity: 82%, positive likelihood ratio: 2.32, and negative likelihood ratio: 0.71. It was also shown that the presence of elongated cells can help predict resistant bacteria more accurately.

**Conclusions:** Follow-up urinary gram-stain is considered useful on treating patients with pyelonephritis as it facilitates evaluation of antibiotic effectiveness at an early stage.

## Background

Urinary infection is one of the most common infections in a clinical setting. The selection of appropriate antibiotics for initial treatment is often difficult. Although the use of broad-spectrum antibiotics reduces the rate of treatment failure, it is desirable to initiate the treatment with narrow-spectrum antibiotics if possible to minimize the risk of developing antibiotic resistance. The effectiveness of antibiotic treatment is evaluated based on the clinical course until the culture results are made available, but there are no concrete criteria to determine the timing of switching antibiotics. It would be markedly beneficial if effectiveness could be evaluated more rapidly and accurately than that based on clinical follow-up of several days. Therefore, we evaluated whether follow-up urinary gram-stain after antibiotic administration can help predict bacterial sensitivity to antibiotics.

## Methods

Patients whose diagnosis at discharge was pyelonephritis from April 1<sup>st</sup>, 2013 to October 8<sup>th</sup>, 2019 in Rakuwakai Marutamachi Hospital were extracted. Ethical approval was granted by the Rakuwakai Marutamachi Hospital Ethics Committee. We analyzed the results of urinary gram-stain before and within 48 hours after the initiation of antibiotic treatment, and drug sensitivity tests of bacteria isolated on urine culture. Patients who had previously been administered antibiotics within 72 hours before the initiation of treatment were excluded. Staining was conducted following Hucker's modification[1, 2]. Only patients positive for gram-negative rods on gram-stain at the first medical examination were included to minimize the influence of indigenous bacteria. If the gram reaction or shapes were variable, evaluation of the effectiveness of antibiotic treatment was conducted only on gram-negative rods. Follow-up gram-stain

was recorded as: remaining, bulge formation, elongation, or disappearance. Bulge formation and elongation were counted as remaining. The results of testing the sensitivity to the antibiotic agent administered were recorded as follows: (S): susceptible, (R): resistant, or (I): intermediate. (I) was considered as (R), as it is likely to lead to a switch of antibiotics in a clinical setting. When more than 1 bacterial species was isolated with the same gram reaction and shape (*E. coli* and *Kl. pneumoniae*, etc.), it was recorded whether the used antibiotic agent was effective against all gram negative bacteria isolated. If one of the isolated bacterial species was (R) or (I), it was recorded as (R) even when the other species was (S). When the antibiotic agent was changed before the follow-up, or more than one antibiotic agent was used, it was considered as resistant only when it was (R) or (I) to all the agents administered, regardless of the number of times administered.

## Results

The study included a total of 271 patients. The mean age was 84.1 (16-101) years old, and 204 of them (72%) were female. Urinary gram-stain was conducted at the first medical examination, and the pathogen was identified with quantitative culture in all cases. In 200 patients, only gram-negative rods were observed on gram-stain at the first medical examination. Follow-up gram-stain was conducted on the day after admission in 263 out of the 271 cases. The respective sensitivity and specificity of follow-up gram-stain (when the cells remained) on predicting resistance were 41 and 82%, and 27 and 83.1% in the group showing only gram-negative rods (Table 1).

Elongation of gram-negative rods was observed in 9 out of the 272 patients on follow-up, and the sensitivity and specificity in those cases were 9.5 (2.3-16.8) and 98.6 (96.9-100)%, respectively (Table 2). Bulging of the cells was not observed.

<b>Table 1. Prediction of antibiotic resistance when the cells remained on follow-up gram-stain</b>					
		Sensitivity	Specificity	LR+	LR-
All cases	n=271	41% (29-53)	82% (77-87)	2.32 (1.53-3.51)	0.71 (0.58-0.89)
Only gram-negative rods*	n=200	27% (12-41)	83% (77-89)	1.60 (0.85-3.02)	0.88 (0.71-1.08)
Note) ( ) shows 95% confidence interval. *gram-stain at the first visit					

<b>Table 2. Prediction of antibiotic resistance when elongation of the cells was observed on follow-up gram-stain</b>			
Sensitivity	Specificity	LR <sup>+</sup>	LR <sup>-</sup>
9.5% (2.3-16.8%)	98.6% (96.9-100%)	6.60 (1.70-25.7%)	0.92 (0.85-1.00%)
Note) ( ) shows 95% confidence interval.			

## Discussion

This study revealed that follow-up gram-stain can be used to predict antibiotic resistance. Furthermore, it was suggested that the possibility of antibiotic resistance is strong when the elongation of gram-negative rods is observed. A number of studies evaluated the usefulness of urinary gram-stain in urinary tract infection. For example, sensitivity and specificity of urinary gram-stain were reported to be 91% (95% CI: 0.86-0.96) and 96% (95% CI: 0.92-0.98) in urinary tract infection of children, respectively, being more favorable than urinalysis and urine sediment examination[3]. Furthermore, a study reported that urinary gram-stain leads to the appropriate choice of antibiotics for the initial treatment of urinary tract infection[4]. However, most previous studies evaluated the usefulness as a diagnostic adjunct, and as far as we searched, there has been no study evaluating the usefulness of gram-stain to predict the effectiveness of antibiotic treatment.

Clinical symptoms such as fever, chills, nausea, and lateral abdominal pain improve within 3 days after appropriate treatment in most cases. On the other hand, 26 and 13% of patients still have an unresolved fever on treatment days 2 and 3, respectively[5]. Therefore, the effect of antibiotics cannot be accurately predicted until the 3<sup>rd</sup> day based solely on the clinical course. Furthermore, physicians must wait to obtain the results of urine culture and antibiotic sensitivity tests, and so follow-up with gram-stain, which can be promptly evaluated, is of marked benefit.

This study suggested that switching to broader-spectrum antibiotics should be considered when the bacterial cells remain on follow-up gram-stain. Especially when elongation is observed, bacteria are more likely to be resistant to antibiotics, and it is a strong basis for a switch. Evaluation of antibiotic effectiveness at an early stage can lead to an improved treatment outcome. In addition, it becomes easier to use narrow-spectrum antibiotics from the initial treatment, and it may encourage switching to oral agents and early discharge, contributing to reductions in both antimicrobial resistance and medical expenditure. On the other hand, the disappearance of cells on follow-up gram-stain should not be the sole basis for effectiveness of antibiotics. It is necessary to evaluate the effectiveness in reference to the clinical course, as conducted conventionally, until the results of urine culture are obtained.

The low sensitivity of follow-up gram-stain for detecting resistant bacteria may be associated with the number of fields of view or timing of follow-up. In this study, the time spent observing and number of such fields were not set, and observation may have been considered sufficient after only a few fields of view in patients with a favorable clinical course. Furthermore, the follow-up timing was set as within 48

hours, and long-term exposure to the antibiotics may have eliminated resistant bacteria. Earlier follow-up may have led to the detection of remaining resistant bacteria.

It was reported that *E. coli* cells undergo gradual elongation, and lysis occurs after approximately 70 minutes when exposed to antibiotics they are sensitive to[6]. Therefore, the presence of elongated cells may be considered to suggest that antibiotics are effective. However, it was also reported that a similar change is observed when cefotaxime-resistant *E. coli* is exposed to cefotaxime[7]. Although elongation itself cannot predict sensitivity or resistance to antibiotics, the continued presence of elongated bacteria after long-term exposure to antibiotics may suggest resistance. In this study, follow-up gram-stain was performed on the day after admission in most cases, following a long period of exposure to antibiotics. As a result, it was considered that the presence of elongated cells at follow-up was associated with a high rate of resistant bacteria.

Limitations of this study include the following. First, as mentioned above, the number of fields of view and amount of time for observation were not set. The favorability of the clinical course may have influenced the observation time and number of fields of view. Secondly, the extraction of cases was based on the final diagnosis on the discharge summary, and patients with disorders such as obstructive pyelonephritis and renal abscess were included. Third, backgrounds of patients were not investigated, and those with urinary retention and urinary catheter insertion were included, possibly generating differences in the degree of drainage of bacteria and urine among cases. Lastly, the timing of follow-up gram-stain was not set. In most cases, it was performed on the day after admission, but the amount of time until follow-up differed depending on the time of admission. The diagnostic accuracy may increase if appropriate follow-up timing is set.

## Conclusion

In conclusion, if remaining or elongated cells are observed on urinary gram-stain conducted within 48 hours after the initiation of treatment, the presence of resistant bacteria should be considered. Further investigation with a set observation time and timing of follow-up is required to improve the diagnostic accuracy.

## Declarations

### **Ethics approval and consent to participate**

This study followed the Declaration of Helsinki. The Rakuwakai Marutamachi Hospital Ethics Committee. (approval number 2020-15) approved this study and waived informed consent because of the retrospective design.

### **Funding**

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

All authors designed the protocol. MA collected the data, wrote the report, and is responsible for the study. All authors analyzed and interpreted the data, reviewed and approved the report.

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The final draft of the paper was proofread by Justin Denley, an English language editor.

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