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Citation	Journal of Infection and Chemotherapy. 2024, 30(8), p. 768-772
Version Type	AM
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**Reviving Sternheimer stain: a single-center retrospective study to detect the
diagnostic utility of urinary tract infections in the emergency department**

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23 **Abstract**

24 **Introduction**

25 Qualitative urinalysis using the Sternheimer stain is a common method in Japan for
26 identifying bacteriuria, but there is a lack of studies examining its test characteristics. In
27 this study, we aimed to investigate the sensitivity and specificity of the Sternheimer
28 stain for urine culture results and compare it with the sensitivity and specificity of the
29 Gram stain. Our goal was to determine the usefulness of the Sternheimer stain in
30 identifying bacteriuria.

31 **Patients and Methods**

32 Among 986 patients aged 16 years or older from whom samples for both urinalysis and
33 urine culture were obtained at the emergency room of Tenri Hospital from January 2019
34 to December 2019, 342 patients with pyuria, defined as the presence of 10 or more
35 white cells per cubic millimeter in a urine specimen, who had not received prior
36 antimicrobial therapy were included. Urine cultures were used for comparison to
37 determine the sensitivity and specificity of Sternheimer and Gram stain in this patient
38 group. A positive Sternheimer stain result was defined as bacteriuria $\geq (1+)$, and that of
39 Gram stain was defined as $\geq 1/1$ field of high-power ($\times 1,000$) oil immersion.

40 **Results**

41 Using urine culture results for comparison, the sensitivity of Sternheimer stain was
42 92.2%, the specificity was 48.5%, the positive likelihood ratio was 1.79, and the
43 negative likelihood ratio was 0.16.

44 **Discussion**

45 Sternheimer stain is a rapid and useful method to exclude bacteriuria in a group of
46 patients with pyuria in the emergency department.

Keywords: Sternheimer stain, Gram stain, pyuria

Introduction

Urinary tract infection (UTI) is one of the most commonly encountered conditions in routine emergency care, with 10% of elderly men and 50% of elderly women reported to have a history of UTI.^{[1][2]} Although UTIs can be easily treated with appropriate antimicrobial agents, untreated UTIs pose a risk of serious complications. Proper diagnosis and selection of antimicrobial agents are necessary to prevent the emergence of resistant bacteria due to antibiotic abuse.^{[3][4]} UTIs are generally known to be accompanied by pyuria, and The Japanese Society of Infectious Diseases / Japanese Society for Chemotherapy (JAID / JSC) "Guidelines for the Treatment of Infectious Diseases 2015" state that pyuria is useful in the diagnosis of urinary tract infections. Urinary tract infection is defined as a bacteriuria of $>10^5$ CFU/mL of a single relevant pathogen in addition to symptoms such as dysuria, residual urine, and fever.^[5]

Pyuria is useful in diagnosing UTIs, but because UTIs are defined by symptoms plus bacteriuria, pyuria alone is not sufficient to confirm the presence of bacteriuria. The definitive test for this is a urine culture. However, diagnosis through culture cannot be confirmed until the bacteria have grown, and there can be no early diagnosis and treatment while waiting for the results of a urine culture.^{[6][7][8][9][10]}

The most useful method for diagnosing urinary tract infections is the Gram stain, which has been reported to have a sensitivity and specificity of 90%. However, in many cases, Gram stain is difficult to perform in the emergency room due to time constraints. It is known that Gram stain takes more than 5 minutes.^{[11][12][13][14]} Therefore, in many cases, the presence of urinary tract infection had been determined from the results of urine qualitative analysis without Gram stain in our hospital.

Sternheimer stain, which was invented in 1975 and is relatively easy to perform, is the most commonly used method for staining urine sediment using a staining solution in many Japanese hospitals.^{[13][14]}

Sternheimer stain is a simple test performed by dropping urine sediment and observing the specimen mixed with a small amount of staining solution, which takes about 1 minute. Sternheimer stain has the advantage of identifying leukocytes and uroepithelial cells, which are difficult to identify in urine sediment without stain.^{[15][16]} In numerous hospitals, including our own, clinical laboratory technicians carry out Sternheimer stain, a test that is feasible by merely applying reagents and observing the specimen. This procedure, standardized by the Japanese Association of Medical Technologists, requires less than one minute to complete.^[17] Furthermore, as clinical laboratory technicians are solely dedicated to specimen testing, they find these examinations more manageable compared to physicians. If the sensitivity and specificity of Sternheimer stain are not inferior to those of Gram stain, Sternheimer stain, with its shorter examination time, may be useful. However, the usefulness of Sternheimer stain for the diagnosis of UTI remains unclear.

The purpose of this study was to investigate the usefulness of Sternheimer stain by examining the sensitivity and specificity of urine qualitative methods and Gram stain when urine culture results are used as the comparison.

Patients and Methods

Study setting and design

This was a single-center, retrospective study. Patients aged 16 years or older from whom samples for urinalysis and urine culture were obtained due to suspected urinary tract infection at Tenri Hospital, an acute care hospital with 715 beds (Tenri city, Nara, Japan) between January 1, 2019, and December 31, 2019, were included in the study. This study followed the Strengthening the Reporting of Observational Studies in

Epidemiology statement for reporting observational studies. ^[18] The Institutional Review Boards of Tenri Hospital approved the study protocol (Number 1373). No written consent was obtained. We have an opt-out document on the hospital website, and all participants have the opportunity to opt out of the use of their data. All information was collected from electronic medical records. All information was collected from electronic medical records. We extracted cases of suspected urinary tract infections from the medical records of emergency room physicians. In our research, UTIs were defined as instances where 1) the presence of microbial organisms was confirmed via urine culture, and 2) a case was diagnosed as a urinary tract infection by a physician, with both elements verifiable through extraction from the medical records. All cases that fell into this category more than once were excluded from the current study because of the possibility of influencing the results. Patients with pyuria and those with a history of antimicrobial therapy up to 3 days before the visit were excluded. On the other hand, patients with urologic disease or indwelling urinary catheters were included in this study. The types of bacteria that were positive in urine culture were also summarized.

Outcome

In this study, the sensitivity and specificity of Sternheimer stain by our clinical laboratory technicians using urine culture as the comparison was determined as the primary outcome and the sensitivity and specificity of Gram staining by a microbiologist as the secondary outcome.

Variable definitions

Urine qualitative methods, Gram staining, and urine culture were performed from intermediate urine, and Sternheimer stain was classified according to bacterial abundance as shown in Figure 1.^[19] Urine culture was considered positive when 10⁵ colony-forming units of uropathogens were detected.

Statistical analysis

To compare the performance of rapid diagnostic tests, the sensitivity, specificity, positive likelihood ratio (LR), and negative LR of each test were calculated. We describe the patients' characteristics using medians and interquartile ranges (IQR) for continuous variables and number and percentage (%) for categorical variables. We used Pearson's chi-square test. All statistical analyses were performed using EZR^[20] version 1.4.1., a modified version of R commander designed to add statistical functions frequently used in biostatistics.

Results

Urine cultures of 986 patients were processed during the study period. Of the 986 patients from whom samples for urinalysis and urine culture were obtained due to suspected urinary tract infection, 627 patients who did not have pyuria (leukocytes \geq 10/HPF in urine sediment) and 17 patients with a history of antimicrobial therapy were excluded, and 342 patients were included in the study to examine the following.^[21] The characteristics of the patients at baseline were well-balanced between the two groups. (Figure 2) Of the remaining 342 patients, 145 (42.4%) were males and the median age was 75.7 years. Background diseases included enlarged prostate and neurogenic bladder

in 34 patients, neoplasms in 28 patients, and diabetes mellitus in 85 patients. (Table 1)

Of these 342 patients, 71% had positive urine culture results.

1) Diagnostic performance of Sternheimer stain

Of the 342 cases, 275 were positive for Sternheimer stain (1+) or more, and 67 were negative for Sternheimer stain (-). Of the Sternheimer stain-positive group, 224 were positive for urine culture and 51 were negative for urine culture, while of the Sternheimer stain-negative group, 19 were positive for urine culture and 48 were negative for urine culture. When urine culture results were used as the comparison, the sensitivity of the Sternheimer stain method was 92.2%, specificity was 48.5%, positive likelihood ratio was 1.79, and negative likelihood ratio was 0.16. (Table 2)

The sensitivity and specificity of the urine qualitative method using Sternheimer stain were 67%, 78.8%, 31.7%, and 89.9%, respectively, when the positivity of the urine qualitative method using Sternheimer stain was set to (2+) \geq and (3+) \geq .

2) Diagnostic performance of Gram stain

Among the 342 cases, 265 cases had more than 1/1 field of view in Gram stain, and 77 cases did not. In the Gram stain-positive group, 243 cases had positive urine culture results, and 2 cases had negative urine culture results. On the other hand, among the Gram-negative group, 24 cases were positive for urine culture and 75 cases were negative for urine culture. (Table 2) When the results of urine culture were used as the comparison, the sensitivity and specificity of Gram stain were 99.2% and 74.7%, respectively, with a positive likelihood ratio of 4.1 and a negative likelihood ratio of

0.01. Of the 243 positive urine culture results, the most common bacteria isolated were *Escherichia coli*, *Klebsiella* spp., and *Enterococcus faecalis*. (Table 3).

Discussion

In this study, we investigated the sensitivity and specificity of the Sternheimer stain and Gram stain methods with urine culture results as the comparison and assessed the usefulness of the Sternheimer stain method.

The sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of the urine qualitative method using Sternheimer Stain in this study were 92.2%, 48.5%, 1.79, and 0.16, respectively. These results suggest that Sternheimer Stain alone is clinically difficult to diagnose UTI, but it is reliable in excluding UTI.

The major advantage of the Sternheimer stain is that it is a very easy and rapid test, and it can exclude UTIs with a high degree of accuracy if it shows absence of pyuria.^[21]

This study suggests that the Sternheimer stain may be a sensitive test method. ~~Gram stain is a highly sensitive and specific test: when 1/HPF bacteria are detected by Gram stain of non-centrifuged specimens, the sensitivity to bacteriuria (10^5 CFU/ml) in quantitative culture is 93% and specificity is 95% in adults.^[20] However, it is not suitable for use in the emergency room because it is time-consuming. In practice, the facilities or skills required to perform Gram staining may not always be available, and the ability to perform Sternheimer staining in a shorter time allows for rapid patient response, which is advantageous.^{[7][10]}~~ Residents also work in emergency rooms in Japan, and it is reported that only 6% of trainees have previously performed Gram stain.^[22] This suggests that many physicians do not routinely perform Gram stain. In

addition, laboratory technicians are not available at night, making it very difficult to perform Gram stain properly. Sternheimer stain serves as an efficacious screening method in the initial detection of urinary tract infections in emergency rooms. In instances where Sternheimer stain yields positive results, it is recommended to proceed with Gram stain. We consider that this sequential approach promotes rapid and precise diagnosis of urinary tract infections and supports a comprehensive microbiological evaluation.

In a previous study of women over the age of 18, the sensitivity and specificity of the urine bacterial count were 83% and 67%, respectively. That study examined the utility of testing for bacteriuria when the gold standard is a positive urine culture. In that study, the prevalence ranged from 40 to 61%, but in the current study, the sensitivity was 92% even in a population with a prevalence close to 70%, suggesting that the test may be more useful in ruling out UTIs than shown in previous studies. ^{[10][11]}

The subjects in this study were a population with a high prior probability of urine culture positivity (71%). Therefore, if bacteriuria could be excluded based on Sternheimer staining, inappropriate antimicrobial administration could be reduced.

There were 19 cases with negative Sternheimer stain and positive urine culture.

However, there were only 6 cases of symptomatic bacteriuria, 5 of which were treated as stone pyelonephritis and 1 as prostatitis. It seems unlikely that symptomatic bacteriuria would be missed in cases with negative Sternheimer stain results.

There are several limitations to this study. First, it is a single-center, retrospective study. Second, the criteria for the Sternheimer stain, which is a semi-quantitative method, are ambiguous, and it is expected that the criteria will vary among institutions. Therefore, external validity may be insufficient. Third, the results may differ from specimen to

specimen due to differences in the evaluation ability of clinical laboratory technicians.

Another limitation is the exclusion of multiple patient groups in the population, but the

number of duplicate cases is small, so the versatility may be high. The Sternheimer stain

is an ambiguous test, but its sensitivity is high and it seems to be useful; a more precise

definition of the Sternheimer stain would further increase its versatility. Prospective

studies with further adjustment for these limitations are warranted.

5. Conclusion

In our study, we found that Sternheimer stain is sensitive in the group of patients with

pyuria in the emergency department. Larger multicenter studies are desirable to

corroborate these studies.

Funding

None

Authorship statement

H.N. and R.M.S. designed the study. H.N. acquired and analyzed data. H.N. drafted the

manuscript. All authors contributed to the writing of the final manuscript, approved its

publication, and agreed to be accountable for all aspects of the work in ensuring that

questions related to the accuracy or integrity of any part of the work are appropriately

investigated and resolved.

Declaration of competing interest

The authors have no conflict of interest.

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316 Table 1: Clinical and laboratory characteristics of this study

317 Table 2: Results of Sternheimer stain and urine culture, Gram stain and urine culture

318 Table 3: Microorganisms isolated from urine cultures

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Table1: Clinical and laboratory characteristics of this study

Characteristic	Sternheimer (+) N=275	Sternheimer (-) N=67	P Value
Age: years: (median (IQR),y)	77.1[66.5,87.9]	69.8[56.4,83.9]	0.93
Sex: male: No. (%)	111(40.4)	34(50.7)	0.12
Urologic diseases other than neoplasma: No. (%)	48(17.5)	7(10.4)	0.047
Urologic neoplasma: No. (%)	20(7.27)	10(14.9)	0.162
Diabetes mellitus: No. (%)	67(24.3)	16(23.9)	0.934
Chronic kidney disease: No. (%)	11(4.0)	4(6.0)	0.480
Steroid user: No.(%)	5(1.8)	3(4.8)	0.204
Cirrhosis: No.(%)	1(0.36)	2(3.0)	0.039

Urological malignancies: Prostate cancer, kidney cancer, bladder cancer, testicular cancer

Urologic diseases other than malignancy: prostatic hypertrophy, overactive bladder, neurogenic bladder

IQR: interquartile range

Table2: Results of Sternheimer stain and urine culture, Gram stain and urine culture

	urine culture (+)	urine culture (-)	
Sternheimer (+)	224	51	275
Sternheimer (-)	19	48	67
	243	99	342
	urine culture (+)	urine culture (-)	
Gram (+)	241	24	265
Gram (-)	2	75	77
	243	99	342

368 Table3: Microorganisms isolated from urine cultures

	Number(%)
<i>Escherichia coli</i>	155(63.7%)
<i>Klebsiella</i> spp.	24(9.9%)
<i>Streptococcus</i> spp.	15(6.1%)
<i>Enterococcus faecalis</i>	11(4.5%)
<i>Proteus mirabilis</i>	8(3.3%)
<i>Pseudomonas aeruginosa</i>	7(2.8%)
<i>Enterobacter</i> spp.	6(2.4%)
Methicillin-Resistant <i>Staphylococcus aureus</i>	5(2.1%)
<i>Aerococcus urinae</i>	2(0.8%)
Others	10(4.1%)

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371 **Figure legends**

372 Figure 1 : Definition of Sternheimer stain

373 Figure 2 : Flowchart for the selection of patients

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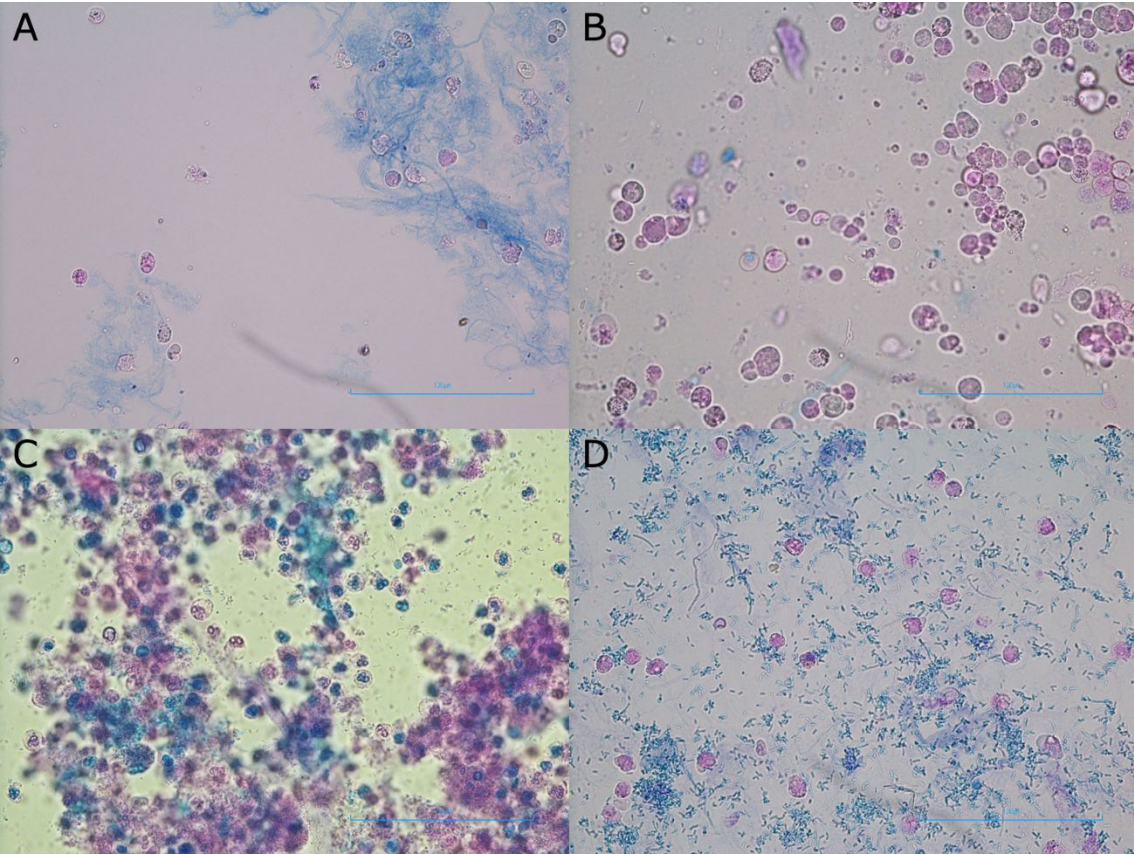
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Figure 1: Definition of Sternheimer stain



A: (-) is noted when only 0 to 9 bacteria are observed in Sternheimer stain. B: (1+) is noted when only 9 to 19 bacteria are observed. C: (2+) is noted when many bacteria are observed. D: (3+) is noted when countless bacteria are observed.

Figure2:Flowchart for the selection of patients

