Original Research

Direct Antibiogram Susceptibility Testing vs Isolation Followed by Subculture in Gram Negative Urine Tract Infections

Hilal Abdessamad[1]*, Omar Jamal[1], Rola Husni[1] and Jacques E. Mokhbat[1]

[1] *Division of Infectious Diseases, Department of Internal Medicine, Gilbert and Rose-Marie Chagoury School of Medicine and the Lebanese American University Medical Center – Rizk Hospital, Beirut, Lebanon.*

*Corresponding Author: Hilal Abdessamad, Division of Infectious Diseases, Department of Internal Medicine, Gilbert and Rose-Marie Chagoury School of Medicine and the Lebanese American University Medical Center – Rizk Hospital, Beirut, Lebanon.

Abstract

Background: Urine tract infections (UTIs) are the main drivers of antimicrobial resistance, especially in gram-negative bacteria. In low-income countries where automized antibiogram determination is not available, there is a need to speed up the identification of resistance profiles of bacteria, which would serve to decrease the days of exposure to broad-spectrum empiric antibiotics.

Methods: We conducted a study to evaluate the feasibility and efficacy of direct identification and antimicrobial susceptibility testing on 50 gram-negative bacterial UTI specimens. We then compared these findings to the standard manual method traditionally used in low-resource settings.

Results: The findings of our study suggest that direct antimicrobial susceptibility testing on urine samples with isolated gram-negative bacilli on gram stain is a reliable tool for rapid susceptibility determination in the vast majority of UTIs. It allowed for determination of an accurate antibiogram within 24 hours of sample collection, which is 24 to 48 hours faster than the standard method.

Conclusion: This approach offers an alternative method of UTI sample handling that can significantly reduce the time to targeted therapy, thereby promoting antimicrobial stewardship and mitigating the growing threat of antibiotic resistance.

Keywords: UTI, Urine, Infection, Identification, Antibiogram

Introduction

Urine tract infections (UTIs) are the most common diseases encountered in any infectious diseases specialty[1]. They are very frequent cases of discomfort and significant morbidity and mortality across various age ranges and risk groups, and across different medical interventions [2]. Whether a simple cystitis in a post-menopausal female, a catheter-associated UTI post-surgery or a recurrent prostatitis in an old male, UTIs pose a significant source of continuous antibiotic use and demand due to their very high incidence [3]. As a result, they are the main driving force of antimicrobial resistance, especially in gram-negative bacteria[3]. In fact, they are the main driver of the Extended-Spectrum Beta-Lactamase (ESBL) pandemic worldwide [4].

The treatment of UTIs consists of two approaches: the empiric approach, guided by local epidemiology and resistance patterns, and the culture-specific approach when the urine culture antibiograms are out[5]. Usually, 18-48 hours are initially required for the initial identification of the causative bacterium, and an additional 24-48h are needed for the emergence of the antibiogram[6]. As a result, the empirical approach remains adopted for up to 4 days before antibiogram-based de-escalation [5].

Due to the high rate of acquisition of resistance to the commonly used antibiotics, every additional day of broad-spectrum antibiotic use poses a risk of increased resistance [7]. For instance, every day of broad-spectrum beta-lactam exposure poses an additional risk of up to 8% of acquisition of new resistance[8,9]. Shortening the duration of exposure to these agents has been shown to decrease the resistance risk[10,11]. This makes it imperative to shorten the duration of exposure to empiric broad-spectrum agents such as piperacillin-tazobactam, cefepime, amikacin or the carbapenems[11,12].

With antimicrobial resistance continuing to rise, rapid identification and direct antibiotic susceptibility testing (AST) poses a reasonable alternative[1,13,14]. Since gram-negative bacteria are the most common agents seen in UTIs, namely *E.coli*, direct gram staining of the centrifuged urine sample concentrate is to be done, and if gram-negative bacilli are seen, direct AST can be done in parallel with the bacterial identification[1]. This can provide a full identification of gram-negative bacteria with the full antibiogram within 2 days rather than 4, providing a safe, accurate and fast guide for rapid de-escalation, limiting further the exposure to the mentioned broad-spectrum agents[3]. This is especially important in low-income countries where automation is not possible.

This project aims to assess the feasibility and efficacy of using direct identification and AST on gram-negative bacterial UTIs in comparison to the standard identification method followed by subculture and AST. The results, if successful, would provide insight about a possible novel approach on microbiological handling of urine samples that can decreased the time of exposure to broad-spectrum agents by at least 2 days.

The project was divided into 2 phases.

- Phase 1 focused on collecting urine specimens and direct gram staining, to identify samples with gram-negative bacteriuria. These samples were directly cultured on a Muller-Hinton agar with the needed antibiogram. At the same time, regular culturing on blood and MacConkey agars commenced in parallel for regular traditional handling.

- Phase 2 compared the results of direct identification and AST vs the standard urine sample handling method.

    a. Group 1 consisting of samples treated with direct identification and AST.

    b. Group 2 consisting of the samples handled traditionally.

Study objectives

To determine feasibility of direct identification and AST.

To decrease the time of identification of resistance patterns to antibiotics.

Clinical importance

This can help decrease the time of de-escalation from empiric to culture-specific antibiotics by at least 2 days.

Study Design

Laboratory experimental study.

Study Population

Urine samples sent to the microbiology laboratory at the Lebanese American University Medical Center – Rizk Hospital

Sampling Design

Each urine sample was handled in parallel by two ways: the standard method and the rapid identification and AST method.

Inclusion Criteria

All urine samples with gram-negative bacilli (GNBs) seen on direct gram stain.

Exclusion criteria

Samples with bacteria seen other than GNBs and contaminated samples (more than 1 type of organism seen)

Methods

Urine samples underwent direct gram staining and those with GNBs were selected.

1. The selected samples were handled as follows

Route 1:

a. Centrifugation and removal of the supernatant

b. Direct performance of Urea-indole-oxidase tests

c. Direct culturing on Muller-Hinton agar and placement of the gram-negative antibiogram: Ampicillin, amoxicillin/ clavulanic acid, piperacillin-tazobactam, cefoxitin, cefixime, ceftazidime, ceftriaxone, cefepime, ertapenem, imipenem, amikacin, gentamycin, tetracycline, trimethoprim-sulfamethoxazole (TMP-SMX), colistin, nitrofu-rantoin, Fosfomycin.

Route 2 (standard of care SoC):

a. Culturing on blood culture and MacConkey agars for 24h

b. Performing the urea-indole-oxidase tests

c. Sub-culturing on the Muller-Hinton agar for the antibiogram

2. Comparison of the results

Results

Fifty samples were included in the study after screening by gram staining and direct microscopy. Of those samples, 38 grew *E. coli* (76%), 5 grew *K. pneumoniae* (10%), 2 grew *P. aerogenosa* (4%), 1 grew *Proteus mirabilis*, 1 grew *Citrobacter freundii*, 1 grew *Enterobacter cloacae* (2% each)and 2 were contaminated samples with Gram positive rods (4%). 1 sample had a growth of pseudomonas on top of *E. coli*. Among the Enterobacteriaceae, 13 were ESBL (28%) producers and 1 was a CRE. 33 samples were given by females (66%) and 17 specimens were given by males (34%).

All samples in the direct antibiogram arm (route 1) had around 4mm less disc diffusion than the SoC arm. However, this did not modify the sample results as it did not affect the qualitative threshold of labeling sensitivity (S) or resistance (R). 45 of the 47 samples had identical qualitative antibiograms and the remaining 2 had the 4mm difference close to the threshold of resistance in 2 antibiotics, one for amoxicillin/clavulanic acid and one for nitrofurantoin. This meant that in this sample, 94% of urine samples can be processed using this method, and 6% would need subculturing. 95% of the samples that could be interpreted yielded identical qualitative results and comparable quantitative disc diffusion measures.

The samples with pseudomonas growth could not be interpreted as the antibiogram used was that for Enterobacteriaceae, where pseudomonas is naturally resistant to many of its components.

Discussion

The data derived from our study shows a very promising alternative to the SoC method of culturing urine samples for diagnosis of a UTI. It offers at least one day less in terms of empirical antibiotic exposure, decreasing the days of exposure to broad spectrum agents, enforcing antimicrobial stewardship programs, and impeding antibiotic resistance. When the urine sample grows pseudomonas, we would also have a hint about fluoroquinolone and aminoglycoside resistance before we go for subcultures. Also, when samples were contaminated with lactobacilli, this method would offer us an effective way to differentiate colonies, a step used in subculture.

Since the data is de-identified, we cannot know whether the samples came from the community or hospital settings. However, the results in this study are consistent with our local epidemiology, where we are expected to see around 25-30% production of ESBL in a random sample of urine specimens[15]. Besides, it coincides with the fact that most of the UTIs we have are seen in females and are caused by *E.coli*, and a small percentage of community acquired UTIs are caused by pseudomonas[15].

The results are laboratory based and not epidemiologic. This means that the SoC arm is considered the true positive and the gold standard, and thus the results in route 2 of the study are all true. The lack of a variation in validity of route 2 results means that we cannot apply statistical methods to determine statistical significance of our results because the essential conditions for hypothesis testing are lacking, which can be a limitation to this study [16,17]. Further application of such results in an epidemiological setting comparing treatment success and failure in patients treated based on these 2 methods would enable us to determine statistical significance. Indeed, such a study serves as evidence that the direct antibiogram method is a good one in urine samples where GNBs were seen on direct microscopy and by such it would set the stone for future development of epidemiologic studies that would test that clinically.

Indeed, in low-resource settings and where there is less reliance on the newer methods of automated antibiogram determination, the direct antibiogram method would be a reliable and a time-effective, cost-effective alternative.

Conclusion

Direct antibiogram testing on urine samples with isolated GNBs seen after gram stain and direct microscopy is a reliable tool to use for rapid susceptibility determination for the vast majority of UTIs in limited resource areas. This would provide a rapid evidence-based reason to switch from empiric to targeted therapy within less time. Such a method would decrease the cost of treatment and the burden of antibiotic resistance, especially in communities where sample handling is not automated.

Ethical Considerations

Samples were handled anonymously. No patient harm or confidentiality breach is present. This study was reviewed and accepted by the Lebanese American University Institutional Review Board.

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