





Perspective

Global Action Against Antimicrobial Resistance (AMR): L-forms, How to Recognize Them Under the Optical Microscope

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Abstract: The Lancet published a systematic review of the global burden of bacterial antimicrobial resistance in 2019, which found a worrying increase. In 2017, World Health Organization (WHO) published a list of priority antibiotic-resistant pathogens, which included the 12 most dangerous bacterial families for human health. We would like to draw attention to the L-forms that are poorly understood at the clinical level in hospital laboratories and have great potential to improve diagnosis and avoid underdiagnosis.

Keywords: Global Antimicrobial Resistance (AMR), forms of resistance *E. coli*, L-forms, urine infection

1. Introduction

In January 2022, The Lancet published the Global Burden of Bacterial Antimicrobial Resistance (AMR), a systematic analysis that estimated 4.95 million of AMR-associated deaths in 2019 [1]. Furthermore, in 2017, World Health Organization (WHO) published a list of priority antibiotic-resistant pathogens, which included the 12 families of most dangerous bacteria to human health. In the critical priority group were multidrug-resistant bacteria that are especially dangerous, with *Escherichia coli* being found in the Enterobacteriaceae group [2].

A previous study published by our group in June 2021 in the Journal of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) diagnosed in vitro investigations of the L-forms found over six years in urinary sediments processed in Vigo health area hospitals (in total, 5760 urine sediments). This perspective article aims to inform laboratory professionals from all hospitals worldwide about the L-forms observed under light microscopy in urinary sediments. Their presence in patients is underdiagnosed, as they are known in the literature but little known in daily hospital practice.

Emmy Klieneberger, in 1935, discovered the L-forms in *Streptobacillus moniliformis* and named them after the Lister Institute where he worked [3]. The vast majority of bacteria have a surrounding cell wall, which gives them shape and mechanical resistance, and both gram-positive and gram-negative bacteria are capable of switching to an “L-form”, a deficient cell wall state [4]. Urinary tract infection (UTI) is nowadays a major health and economic problem, especially in elderly patients with associated comorbidities. In these susceptible patients, the presence of cell wall deficient bacteria, L-form is found in the fresh urine of these patients. The shift to the L-form is a physiologically relevant phenomenon that contributes to the recurrence of infection in older patients with UTI [5-8].

L-forms have been detected in urine samples by filtration and observed by phase contrast microscopy [9]. Numerous reports from clinical laboratories indicate that it can be important to move to an L-form and back to a walled state [10]. These bacterial forms are a public health problem as they are difficult to eradicate, forming a resistance against cell wall-acting antibiotics treatment (especially beta-lactam antibiotics) and can survive in vivo despite their defective cell wall [11-15].

Due to their polymorphic nature, it is very easy to confuse L-forms with structures of eukaryotic origin, such as granules, apoptotic bodies or other membrane vesicles, which are especially abundant in diseased tissues [5]. The importance of recognizing these resistance forms under the light microscope lies in the fact that they can easily be confused with other elements in the urinary sediment of patients, especially with yeasts [16], and if the observer is not familiar with them when the study is performed in hospital clinical laboratories.

Under the light microscope, they show a morphology with exaggerated elongations and the presence of lumps and deformations, due to the total or partial loss of their cell wall (Figures 1-4). If these L-forms are suspected in the patient's urine sediment, a confirmatory diagnosis should be made by a urine culture to alert the clinician to the presence of antibiotic-resistant bacteria whose mechanism of action acts on the cell wall, thus facilitating the change of antibiotic by the clinician and taking this into account for subsequent antibiotic treatment, to avoid an increase in bacterial resistance and possible deaths due to AMR. Therefore, it is essential for hospital clinical laboratory professionals to be aware of these L-forms in the urine sediment of patients under the light microscope.

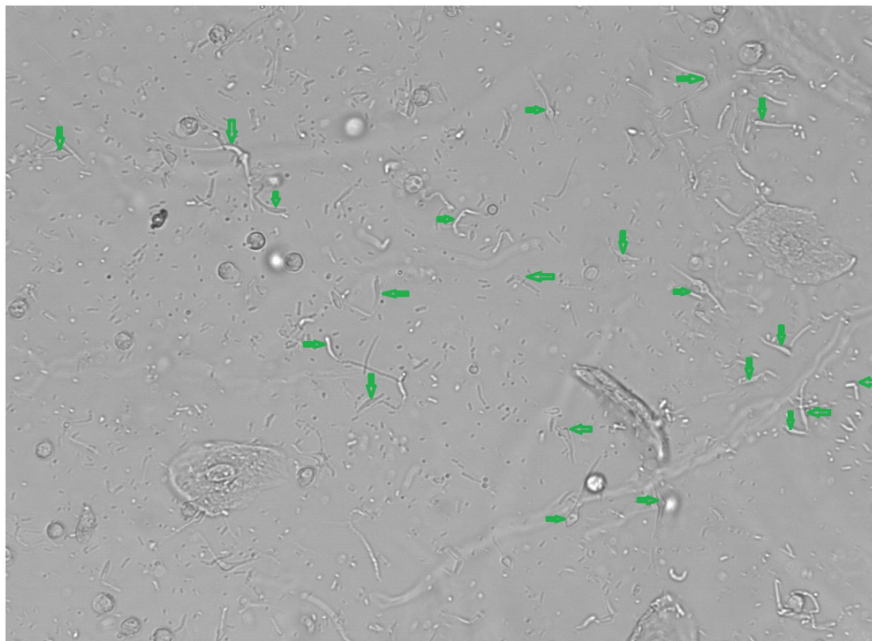


Figure 1. L-forms seen under the light microscope (marked with green arrows)

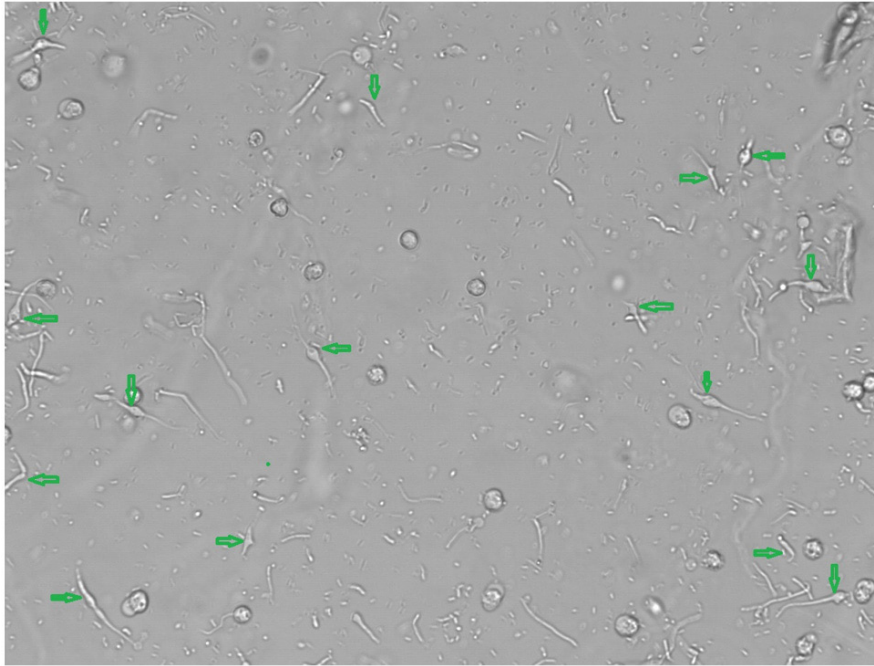


Figure 2. L-forms seen under the light microscope (marked with green arrows)

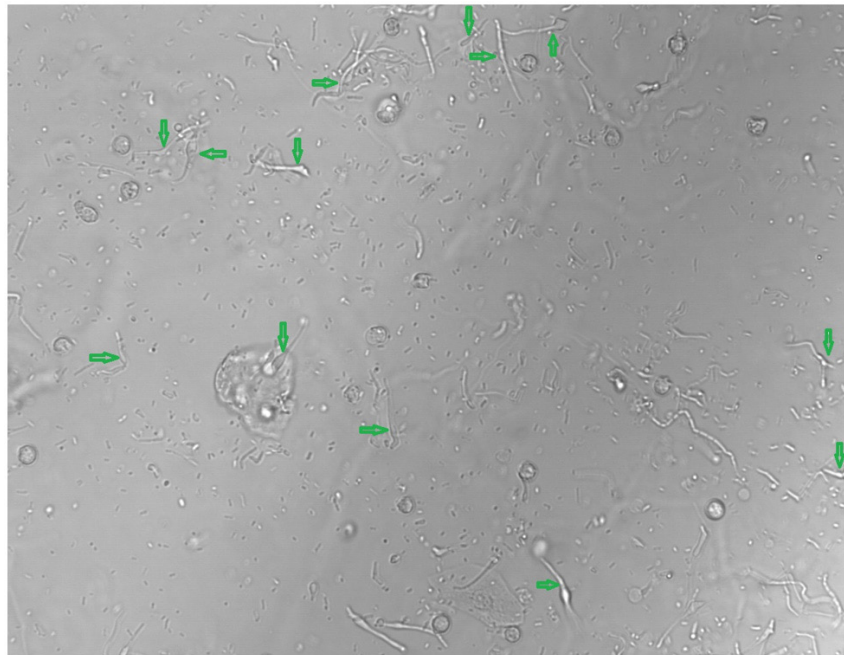


Figure 3. L-forms seen under the light microscope (marked with green arrows)

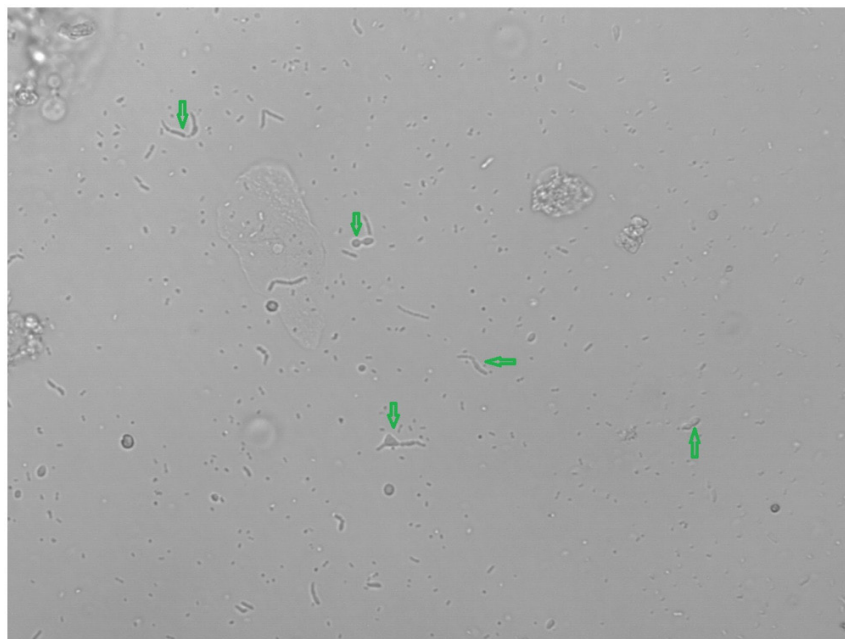


Figure 4. L-forms seen under the light microscope (marked with green arrows)

2. Methods

We investigated prospectively for 6 years, all the L-forms were found in urinary sediment in the urine area of the hospital's laboratory in the health area of Vigo, Spain. The urine samples were analyzed by optical microscopy and a total of 5 L-forms of microbial resistance were found, with a single type of bacteria, *E. coli*. These patients, all of them women, had been previously treated with beta-lactam antibiotics, specifically with amoxicillin plus clavulanic acid in four of them and the other was treated with the antibiotic fosfomycin. Once these forms are detected, the hospital clinician is informed of the presence of these L-forms and advised to discontinue treatment with antibiotics whose mechanism of action acts on the bacterial wall.

3. Results

When the clinical laboratory technicians alerted to the suspicion of L-forms in the sediment visualized under the light microscope, following the section protocol, they seeded the urine on a BD CHROMagar Orientation Medium plate (Beckton Dickinson, New Jersey), which is a selective non-chromogenic medium for direct identification and differentiation of urinary tract pathogens. After seeding, the plates were stored in an oven at 37 °C and growth was observed the following day. With this presumptive diagnosis, the laboratory report alerting the hospital physician to the presence of L-forms was sent. Subsequently, a urine culture was performed in the Laboratory Department of University Hospital of Vigo, Spain, which confirmed the diagnosis, finding a single pathogen in all of them: the bacterium *E. coli*.

We found these forms of resistance in a total of 5760 pathological urine sediments, in none of them, showed yeast growth, which is the most frequent form of resistance that can be confused. All of them were gram-negative bacteria and only one germ grew, *E. coli*, one of the most dangerous germs, where we are running out of antibiotics due to bacterial resistance to antimicrobials. No case was negative because the technicians who alerted of the suspicion of these forms of resistance were correct in all of the cases.

The advantage of recognizing these L-forms in the urine sediment is that it advances the diagnosis of these forms of resistance. In this type of analysis, the experience of the observer is important, he/she needs to know them beforehand, as it enables him/her to recognize and identify them as early as possible.

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