Computers in Biology and Medicine 67 (2015) 104–115

Contents lists available at ScienceDirect

Computers in Biology and Medicine

journal homepage: www.elsevier.com/locate/cbm

Development of an automatic identification algorithm for antibiogram analysis

Luan F.R. Costa [a], Eduardo S. da Silva [b], Victor T. Noronha [b], Ivone Vaz-Moreira [b,c], Olga C. Nunes [b], Marcelino M. de Andrade [a,*]

[a] LIPIS – Laboratory of Instrumentation, Image and Signal Processing, Gama Campus, University of Brasília, Brazil
[b] LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Portugal
[c] CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Portugal

ARTICLE INFO

Article history:
Received 27 June 2015
Accepted 28 September 2015

Keywords:
Antibiogram
Automatic measuring
Discs labels
Inhibition zone
Disc diffusion method

ABSTRACT

Routinely, diagnostic and microbiology laboratories perform antibiogram analysis which can present some difficulties leading to misreadings and intra and inter-reader deviations. An Automatic Identification Algorithm (AIA) has been proposed as a solution to overcome some issues associated with the disc diffusion method, which is the main goal of this work. AIA allows automatic scanning of inhibition zones obtained by antibiograms. More than 60 environmental isolates were tested using susceptibility tests which were performed for 12 different antibiotics for a total of 756 readings. Plate images were acquired and classified as standard or oddity. The inhibition zones were measured using the AIA and results were compared with reference method (human reading), using weighted kappa index and statistical analysis to evaluate, respectively, inter-reader agreement and correlation between AIA-based and human-based reading. Agreements were observed in 88% cases and 89% of the tests showed no difference or a < 4 mm difference between AIA and human analysis, exhibiting a correlation index of 0.85 for all images, 0.90 for standards and 0.80 for oddities with no significant difference between automatic and manual method. AIA resolved some reading problems such as overlapping inhibition zones, imperfect microorganism seeding, non-homogeneity of the circumference, partial action of the antimicrobial, and formation of a second halo of inhibition. Furthermore, AIA proved to overcome some of the limitations observed in other automatic methods. Therefore, AIA may be a practical tool for automated reading of antibiograms in diagnostic and microbiology laboratories.

1. Introduction

Microbiologists play an important role in identifying the drugs that will be most effective in the treatment of clinical infections, as well as in defining the antibiotic resistance profiles of microorganisms found in the environment. Such environmental microorganisms have been established as antibiotic resistance disseminators [1–5]. Therefore, it is necessary to evaluate the sensitivity of microorganisms to antimicrobial agents as quickly as possible once isolated. Similarly, clinical determinations of the antibiotic resistance profile of a pathogen are critical for correct treatment.

There are different types of susceptibility tests available, including disc diffusion and broth micro-dilution methods, as well as commercial tests for the determination of the minimal inhibitory concentration (MIC). The disc diffusion method is the most commonly used method worldwide [6], mainly owing to its low cost and simplicity. In this method, each disc containing an antimicrobial agent will form an inhibition zone where the microorganism is not able to grow. The size (diameter) of the inhibition zone is used to classify the strains as resistant (*R*), intermediate (*I*), or sensitive (*S*) [7].

Several organizations are responsible for regulating the standardization of susceptibility tests, procedures, and interpretation criteria: the United States Food and Drug Administration (FDA), the Clinical and Laboratory Standards Institute (CLSI), the National Committee for Clinical Laboratory Standards (NCCLS), and the French Society for Microbiology (SFM) [7]. These standards help to define the threshold diameters defining the antibiotic resistance phenotype (*R*, *I*, and *S*), tables for MIC interpretative criteria, as well as the optimal method for the preparation of the inoculums and the indication of which antimicrobials should be tested for each microorganism [6,7]. The spatial arrangement of antimicrobial discs on the

* Corresponding author.
E-mail addresses: luanfrc@gmail.com (L.F.R. Costa), eduardossilva06@gmail.com (E.S. da Silva), victor.tn@outlook.com (V.T. Noronha), ivone@fe.up.pt (I. Vaz-Moreira), opnunes@fe.up.pt (O.C. Nunes), andrade@unb.br (M.M.d. Andrade).

http://dx.doi.org/10.1016/j.compbiomed.2015.09.020
0010-4825/

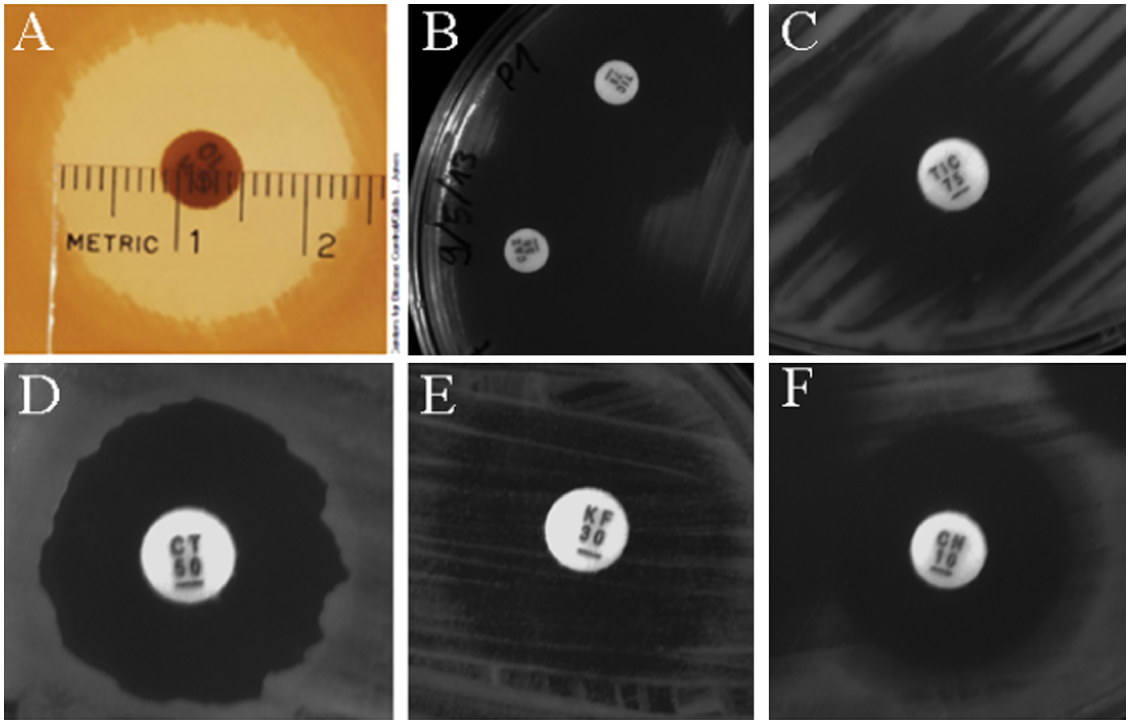

Fig. 1. Automatic identification algorithm (AIA) fluxogram. (A) Millimeter-scale ruler [9]. (B) Overlapping of inhibition zones. (C) Problems related with the seeding of the organism. (D) Non-homogeneous circumference. (E) Partial action of the antimicrobial. (F) Second inhibition halo.

plates is not always standardized and is usually defined by the professional in charge. However, strategies such as the use of a disc dispenser can help to standardize this procedure and simplify the task, mainly to standardize the discs in the same position when there is the need for numerous antibiograms. Nonetheless, a critical aspect of this procedure is the antibiogram reading step, after all while the manual zone measurement is reliable, the use of automated approach can reduce the number of errors and improve the accuracy of susceptibility test [8].

The measurement of the inhibition zone diameter is usually performed manually by specialists using a millimeter-scale ruler (Fig. 1A). Although seemingly trivial, this task may present several challenges such as overlapping of inhibition zones [9-12] (Fig. 1B), problems related with the seeding of the organism [13] (Fig. 1C), non-homogeneity of the circumference (Fig. 1D), partial action of the antimicrobial (Fig. 1E), and formation of a second inhibition halo [14,15] (Fig. 1F). When faced with any one of these challenges, accurate interpretation of the results is dependent on the experience of the professional. In addition, the manual measurement of inhibition zones can take a considerable amount of time, making the method impractical for some diagnostic laboratories, mainly those in hospitals that must run through several samples in a timely manner. One potential solution proposed thus far is to focus effort on developing new methods for the automatic interpretation of susceptibility tests.

An earlier approach [16] presented a solution for automatic identification of inhibition zones; however, this solution did not provide strategies to avoid problems such as overlapping and non-homogeneity of the inhibition zones. Another report [17] approached this challenge through the detection of edges with respect to their texture; meanwhile, this method is based only on the saturation of pixels for locating the discs. Another proposed approach for automatic identification of inhibition zones [13] relies on the assumption that only the regions of inhibition are homogeneous, which is not always true. Furthermore, Legrand et al. [18] presented an automatic method but did not describe the image processing techniques there used. The Oxoid Aura Image System [19] shows promising results; nevertheless, the techniques used are also not fully disclosed, which prevents the reproduction and application of the algorithm. Nevertheless, the main goal of these methods is to simplify and accelerate the processing of antibiograms, as well as their reading and interpretation, and to avoid variations in intra- and inter-observer readings when manually measured [13–18].

Toward this end, the aim of the present study was to develop and detail an automated method for the detection of inhibition zones that can overcome the challenges described above to allow for simple readings of antibiograms obtained through the disc diffusion method. The measurements provided by an image-processing Automatic Identification Algorithm (AIA) were compared with those obtained by simultaneous manual measurements of the inhibition zones, performed by a professional using a ruler. A set of 63 environmental strains was used for this comparison.

2. Material and methods

2.1. Bacterial isolates and antibiotics

In this study, a set of 63 environmental isolates, recovered from different aquatic habitats, were analyzed for susceptibility to antibiotics by a routine process described by Ferreira da Silva et al. [20], using Mueller–Hinton agar (Oxoid Limited; Hampshire, UK) in 90 × 15-mm Petri dishes. For each isolate, the susceptibility tests were performed for 12 different antibiotics: amoxicillin (25 μg), gentamicin (10 μg), ciprofloxacin (5 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), tetracycline (30 μg), cephalothin (30 μg), meropenem (10 μg), ceftazidime (30 μg), ticarcillin (75 μg), colistin sulfate (50 μg), sulfamethoxazole (25 μg), and streptomycin (10 μg). In each plate, six discs were manually applied with a disc dispenser (Thermo Scientific™ Oxoid™ Antimicrobial Susceptibility Disk Dispenser, ST6090, Waltham, MA,