

Antibiogramj: A tool for analysing images from disk diffusion tests



C.A. Alonso^a, C. Domínguez^b, J. Heras^{b,*}, E. Mata^b, V. Pascual^b, C. Torres^a, M. Zarazaga^a

^a Biochemistry and Molecular Biology Area, University of La Rioja, Ed. Científico Tecnológico-CCT. C/ Madre de Dios, 53, Logroño, 26006, Spain

^b Department of Mathematics and Computer Science, University of La Rioja, Ed. Científico Tecnológico-CCT. C/ Madre de Dios, 53, Logroño, 26006, Spain

ARTICLE INFO

Article history:

Received 18 November 2016

Revised 6 February 2017

Accepted 9 March 2017

Keywords:

Antibiograms

Inhibition zone reader

Disk diffusion test

ImageJ

OpenCV

Learning system

ABSTRACT

Background and objectives: Disk diffusion testing, known as *antibiogram*, is widely applied in microbiology to determine the antimicrobial susceptibility of microorganisms. The measurement of the diameter of the zone of growth inhibition of microorganisms around the antimicrobial disks in the *antibiogram* is frequently performed manually by specialists using a ruler. This is a time-consuming and error-prone task that might be simplified using automated or semi-automated inhibition zone readers. However, most readers are usually expensive instruments with embedded software that require significant changes in laboratory design and workflow.

Methods: Based on the workflow employed by specialists to determine the antimicrobial susceptibility of microorganisms, we have designed a software tool that, from images of disk diffusion tests, semi-automatises the process. Standard computer vision techniques are employed to achieve such an automation.

Results: We present *Antibiogramj*, a user-friendly and open-source software tool to semi-automatically determine, measure and categorise inhibition zones of images from disk diffusion tests. *Antibiogramj* is implemented in Java and deals with images captured with any device that incorporates a camera, including digital cameras and mobile phones. The fully automatic procedure of *Antibiogramj* for measuring inhibition zones achieves an overall agreement of 87% with an expert microbiologist; moreover, *Antibiogramj* includes features to easily detect when the automatic reading is not correct and fix it manually to obtain the correct result.

Conclusions: *Antibiogramj* is a user-friendly, platform-independent, open-source, and free tool that, up to the best of our knowledge, is the most complete software tool for *antibiogram* analysis without requiring any investment in new equipment or changes in the laboratory.

© 2017 Elsevier B.V. All rights reserved.

1. Background

Determining the antimicrobial susceptibility of microorganisms is of great importance in clinical microbiology not only to guide therapeutic decisions in infectious diseases, but also to investigate the evolution and epidemiology of resistance [1] – which is essential for implementing hospital prevention programs. Antimicrobial susceptibility tests are used to examine the *in vitro* activity of different antimicrobial agents against a specific microorganism. There are a variety of methods for determining antimicrobial susceptibility, including disk diffusion, agar dilution or broth microdilution. All these techniques must be performed under standardised conditions (culture media, temperature, incubation time, among others) in order to achieve assessable, interpretable, reproducible and comparable test results.

The Kirby-Bauer or disk diffusion assay, also known as *antibiogram*, is a qualitative test widely used in routine microbiology practice, mainly in bacterial isolates, due to its convenience, reliability and low cost [1,2]. In this method, cellulose disks impregnated with specific dilutions of different antimicrobial agents are placed on the surface of agar plates previously inoculated with a standardised suspension of the microorganism [3]. Agar plates are incubated using optimal conditions depending on the microorganism, and, then, the diameter (in mm) of the zone of growth inhibition surrounding each antimicrobial disk is measured. The diameter of the inhibition zone is related to the susceptibility of the isolate and to the diffusion rate of the antimicrobial through the agar medium [4]. Hence, this allows the categorisation of the bacterial isolate as susceptible, intermediate or resistant to each antimicrobial drug tested according to the clinical breakpoints established by international committees such as the Clinical and Laboratory Standards Institute (CLSI) [5] or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [6]. In the first case, breakpoints are published annually, whereas in the

* Corresponding author.

E-mail address: jonathan.heras@unirioja.es (J. Heras).

second case, they are permanently available and updated annually on its website (<http://www.eucast.org/>).

Some antimicrobial agents may appear active *in vitro* due to the weak expression of certain resistance mechanisms, but are not effective clinically. Thus, it is important to carry out an interpretative reading of the antibiogram as a complement of the susceptibility test results. This global analysis of a specific susceptibility pattern may lead to the modification of inconsistent clinical classifications and the prediction of susceptibility values of other drugs not included in the antibiogram [7,8].

The measurement of the diameter of the zone of growth inhibition in the antibiogram obtained by disk diffusion test is frequently performed manually by specialists using a ruler. Subsequently, the specialist consults the standard's breakpoints and categorises the bacterial isolate for each drug tested as susceptible, intermediate or resistant. This process is time-consuming and the task of measuring the inhibition zones can be highly dependent on the researcher. Automated and semi-automated inhibition zone readers facilitate objective and rapid resulting, reduce the operator variability in reading plates and also reduce the likelihood of introducing errors in the transcription of results [9]. In addition, these systems enable a more thorough quality review, and facilitate the comparison of cultures from multiple sites and at multiple time points during incubation [10,11]. However, automated inhibition zone readers are usually expensive instruments with embedded software that require significant changes in laboratory design and workflow [11].

An affordable alternative to those systems could be software packages that deal with images of agar plates from disk diffusion tests (from now on, plate-images) captured with a camera device. Nevertheless, there are just a few standalone programs for analysing plate-images, and they lack some key features such as: an easy-to-use interface, a database to store the analysed images, or integration with the standards' breakpoints.

In this paper, we present *AntibiogramJ*, a user-friendly software tool to semi-automatically determine, measure and categorise inhibition zones of plate-images. *AntibiogramJ* deals with images captured with any device that incorporates a camera, including digital cameras and mobile phones. *AntibiogramJ* is an open-source, platform-independent and free tool. In addition, *AntibiogramJ* incorporates key features that help in the analysis of plate-images (e.g. an integrated database, the visualisation of breakpoints on the images, the chance of loading different standards, and so on). Therefore, *AntibiogramJ* provides the benefits of automated and semi-automated inhibition zone readers but reducing costs – since no special equipment is needed – and changes in the laboratory.

2. Implementation

AntibiogramJ has been developed as a Java application. It relies on two third-party libraries widely applied in bioinformatics: *ImageJ* [12], that provides functionality for image processing, and *OpenCV* [13], that features several computer vision and machine-learning algorithms. The combination of those two libraries was possible thanks to the *IJ-OpenCV* library (<http://joheras.github.io/IJ-OpenCV/>). Additionally, *AntibiogramJ* includes an embedded database provided by the *JavaDB* library [14].

There are three main concepts in *AntibiogramJ*: *plate*, *antibiogram* and *experiment*. A *plate* corresponds to the determination, measurement and categorisation of the inhibition zones of one plate-image (such an image might have been captured using non-specialised devices like digital cameras and mobile phones). An *antibiogram* collects the data from several plates inoculated with the same microorganism. Finally, an *experiment* gathers antibiograms. These three concepts are integrated in the user-friendly graphical-user-interface of *AntibiogramJ* and persist in the program using

an embedded *JavaDB* database (the structure of the *AntibiogramJ* database is provided as a supplementary material). The interface of *AntibiogramJ* has been designed to smooth its learning curve, and it guides the user by means of metaphors, tooltips, autocomplete fields, wizards, and enabling/disabling functionality when needed.

The *AntibiogramJ* main window (see Figs. 1–3) consists of 3 graphical entities. The *AntibiogramJ* menu provides the functionality to manage experiments; namely, it allows the user to create a new experiment, open and close experiments, generate a new experiment by using antibiograms of other previously created experiments, and import an experiment from a file – the latter feature allows the user to share experiments across computers using the export functionality included in *AntibiogramJ*. In addition, from this menu, the user can manage standards (see Section 2.1), and make searches (see Section 2.3). The *experiment panel* (left side of the interface) contains the active experiment, its associated antibiograms, and the plates that form such antibiograms. From this panel, the user can incorporate plates to the experiment: if the plate was inoculated with one bacterial isolate already present in one of the antibiograms of the experiment, the plate is included in such an antibiogram; otherwise, a new antibiogram containing the plate is created and added to the experiment – in both cases, the process is carried out automatically by *AntibiogramJ*. In addition, from the experiment panel, the user can remove antibiograms and plates from the experiment, export all the information of the experiment and its antibiograms to an Excel file, and export the experiment to a file, that might be shared to other researchers and later imported to *AntibiogramJ*. Finally, the *tabbed panel* shows the information acquired in the process of reading the plates that form the antibiograms of the experiment (see Section 2.2).

The rest of this section is devoted to present the key features of *AntibiogramJ*.

2.1. Antimicrobial susceptibility testing standards

Before categorising the inhibition zones of plate-images, it is necessary to establish the standard that will be employed for such a categorisation. As we have indicated in the Introduction, there are several organisations that regulate the standardisation of susceptibility tests, procedures, and interpretation criteria (e.g. CLSI, or EUCAST). By default, the current version of *AntibiogramJ* works with the EUCAST standard v.6.0 [6]. However, some laboratories might work with other standards – for instance, CLSI – and, it might be necessary to update the version of some standards – for instance, new versions of the EUCAST standard are released annually. In order to deal with this issue, *AntibiogramJ* provides the functionality to load other standards, or new versions of them, and select the standard to use.

It is worth mentioning that each standard has its own format; hence, hard-coding the transformation of each of them to the internal representation of *AntibiogramJ* is not suitable because it means that the *AntibiogramJ* code should be altered to deal with the different standard formats and their versions. Instead, we employ a new format, called *AntibiogramJXML*, as an intermediate step in the conversion process.

The *AntibiogramJXML* format is based on the XML (eXtensible Markup Language) format and is therefore independent of any particular computer system and extensible for future needs. The structure of XML files following the *AntibiogramJXML* format is fixed by an XML Schema [15], that not only determines the structure of XML files but also specifies and restricts the content of their elements – the *AntibiogramJXML* Schema is provided in the supplementary materials. This schema was developed taking into account the information that is needed to determine the susceptibility of bacteria to the different antimicrobials. *AntibiogramJXML* files are structured in two main blocks: metadata and breakpoints'

Summary of experiment: Experiment 1

E. coli 78250940

Antimicrobial	Code	Category	Diameter (mm)	Microcolony	Interpretation	Standard
Ciprofloxacin-5	CP 5	Susceptible	28.2			EUCAST (v. 6.0)
Amikacin-30	AK 30	Intermediate	17.3			EUCAST (v. 6.0)
Gentamicin-10	GA 10	Susceptible	18.4			EUCAST (v. 6.0)
Tobramycin-10	TOB 10	Intermediate	16.5			EUCAST (v. 6.0)
Trimethoprim-sulfamethoxazole-1	SXT 25	Resistant	6.0			EUCAST (v. 6.0)
Nalidixic acid-30	NA 30	Not available	19.8			EUCAST (v. 6.0)
Ampicillin-10	AMP 10	Resistant	6.0			EUCAST (v. 6.0)
Amoxicillin-clavulanic acid-20-10	AMC 20	Resistant	6.0			EUCAST (v. 6.0)
Cefotaxime-5	CTX 10	Resistant	6.4			EUCAST (v. 6.0)
Cefotaxime-30	CTX 30	Resistant	17.4			EUCAST (v. 6.0)
Ceftazidime-10	CAZ 10	Resistant	17.0			EUCAST (v. 6.0)

S. sclari 290

Antimicrobial	Code	Category	Diameter (mm)	Microcolony	Interpretation	Standard
Cefotaxime-30	CTX 30	Resistant	21.5			EUCAST (v. 6.0)
Ciprofloxacin-5	CP 5	Susceptible	24.5			EUCAST (v. 6.0)
Gentamicin-10	GA 10	Susceptible	30.5			EUCAST (v. 6.0)
Tobramycin-10	TOB 10	Susceptible	30.0			EUCAST (v. 6.0)
Trimethoprim-sulfamethoxazole-1	SXT 25	Resistant	23.5			EUCAST (v. 6.0)
Benzalkonium chloride-1	B 15	Not available	22.0			EUCAST (v. 6.0)
Tetracycline-30	TE 30	Susceptible	31.0			EUCAST (v. 6.0)
Clindamycin-2	DA 2	Not available	14.0			EUCAST (v. 6.0)

Fig. 1. Main window of AntibioGramJ showing a summary of the active experiment.

Summary of experiment: Experiment 1

E. coli 78250940

Microbial group	Genus	Species	# Strains	Sample	Antimicrobial	Code	Category	Diameter (mm)	Microcolony	Interpretation	Standard
Bacterial isolate	Escherichia	E. coli	125		Ciprofloxacin-5	CP 5	Susceptible	28.2			EUCAST (v. 6.0)
					Amikacin-30	AK 30	Intermediate	17.3			EUCAST (v. 6.0)
					Gentamicin-10	GA 10	Susceptible	18.4			EUCAST (v. 6.0)
					Tobramycin-10	TOB 10	Intermediate	16.5			EUCAST (v. 6.0)
					Trimethoprim-sulfamethoxazole-1	SXT 25	Resistant	6.0			EUCAST (v. 6.0)
					Nalidixic acid-30	NA 30	Not available	19.8			EUCAST (v. 6.0)
					Ampicillin-10	AMP 10	Resistant	6.0			EUCAST (v. 6.0)
					Amoxicillin-clavulanic acid-20-10	AMC 20	Resistant	6.4			EUCAST (v. 6.0)
					Cefotaxime-5	CTX 10	Resistant	6.4			EUCAST (v. 6.0)
					Cefotaxime-30	CTX 30	Resistant	17.4			EUCAST (v. 6.0)
					Ceftazidime-10	CAZ 10	Resistant	17.0			EUCAST (v. 6.0)
					Chloramphenicol-30	C 30	Susceptible	22.8			EUCAST (v. 6.0)
					Tetracycline-30	TE 30	Not available	31.0			EUCAST (v. 6.0)

Fig. 2. Main window of AntibioGramJ when an antibiogram of the active experiment is selected.

data. The former gives general information about the standard like the name, the version and the validity. The latter contains information about the breakpoints for the different microbial groups and antimicrobials. Files that follow the AntibioGramJXML format can be nicely visualised in any web browser thanks to an XSLT (eXtensible Stylesheet Language Transformations) file [15] – an example is provided in the supplementary materials.

The greatest advantage of using the AntibioGramJXML format is that it allows AntibioGramJ to deal in an elegant manner with different standards. Instead of hard-coding the conversion in AntibioGramJ, an external program is employed to convert the standard in its original format to the AntibioGramJXML format – such an external program might be created not only by the developers of AntibioGramJ but also by third-parties. Subsequently, the generated file can be loaded and used in AntibioGramJ. In this way, new standards can be easily incorporated into AntibioGramJ.

Once the desired standard is selected, the user can create an experiment and add antibiograms to it by means of the *plate analyser wizard*.

2.2. The plate analyser wizard

AntibioGramJ provides a wizard to analyse plate-images – AntibioGramJ supports the most common standard image-formats including tiff, jpeg, png, gif, and bmp. As a result of the analysis, a plate is added to either a new or an existing antibiogram of the active experiment. The plate analyser wizard guides the user through the 5 steps that are required to analyse a plate-image: bacterial isolate data, image pre-processing, antimicrobial disk management, inhibition zone reading, and inhibition zone annotation (see Fig. 4).

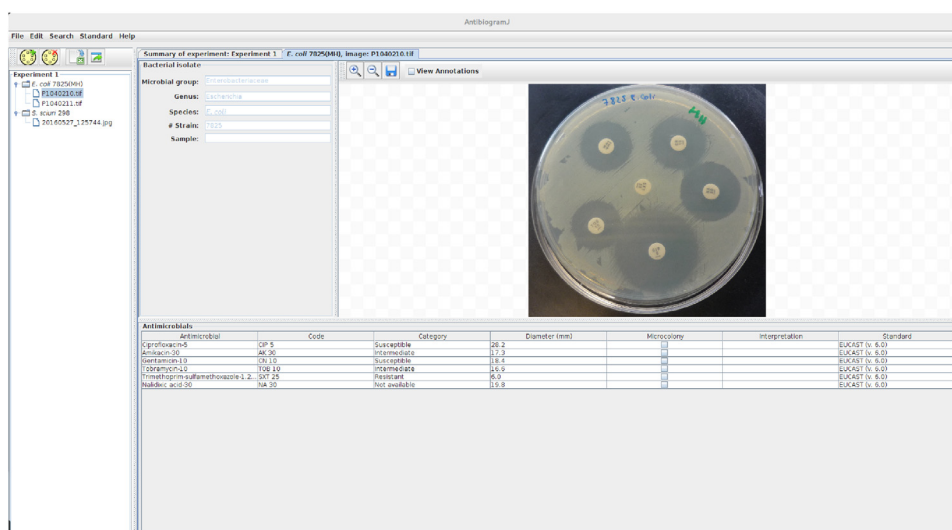


Fig. 3. Main window of AntibioGramJ when a plate of one of the antibiograms of the active experiment is selected.

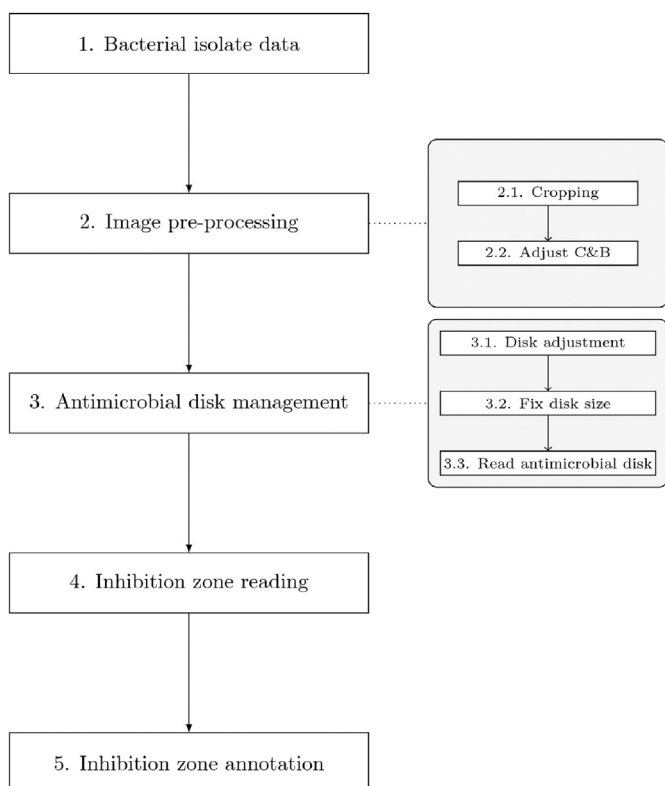


Fig. 4. Workflow of the plate analyser wizard. Left. Main steps of the plate analyser wizard – even if the flow of the figure goes from top to bottom, the user can go back at any point. Right. Substeps of each step of the plate analyser wizard.

Step 1. Bacterial isolate data. In the first step, the users select whether they want to add the plate to an antibiogram of the experiment, or if they want to create a new antibiogram by introducing the information about the analysed bacterial isolate. In particular, the data that must be provided are the microbial group, genus, species, and strain number of the bacterial isolate; and, optionally, the sample of the bacterial isolate and some comments. These data are introduced by means of autocomplete text fields that remember the information that was introduced previously for other bacterial isolates.

Step 2. Image pre-processing. Since AntibioGramJ does not restrict the devices, or the conditions, used to capture plate-images, there is a huge variability on those images. Therefore, it might be necessary to adjust the quality of the images using the functionality provided by AntibioGramJ. First of all, the user can crop the image to keep in the image just the plate; this action can be performed either manually or automatically – the automatic cropping is carried out using the OpenCV library; namely employing the Hough circle transform [16]. Moreover, the user can adjust, both manually and automatically, the brightness and contrast of the plate-image – this functionality is provided by the ImageJ library.

Step 3. Antimicrobial disk management. AntibioGramJ automatically detects the disks in the plate-images and reads the codes of the antimicrobials written in those disks. In order to achieve this functionality, the first step is a combination of filtering and thresholding that is applied to automatically detect the white disks of the image. In some situations (e.g. if the quality of the image is low), this automatic detection might need some adjustments – namely, the user can add and remove disks, and adjust their position and size. Subsequently, the user must indicate the size in millimeters of one of the diameters of the detected disks, by default this value is 6mm. This information is employed to calibrate the scale of the image – a step that allows the use of images captured with different devices, that might have different lenses and image sensors, and at different distances.

Finally, the codes written in the antimicrobial disks are read, and for each code the corresponding antimicrobial is associated. This reading of the antimicrobial codes is performed by means of a *learning system* that matches the disks against previously seen disks with the same code. Instead of using an OCR (Optical Character Recognition) to read the letters written in the disks, we use this matching procedure because different providers use different codes for the same antimicrobial (for instance, Oxoid uses AK as the code for amikacin, whereas BD uses the code AN); hence, AntibioGramJ adapts itself to the antimicrobial disks employed in each laboratory. It is worth noting that, in this approach, the first times that the users employ AntibioGramJ, they need to teach the system, by selecting from a list of antimicrobials; and such a selection is learned for future use. In the matching procedure, the learning system employs the ORB binary descriptor [17] provided by OpenCV.

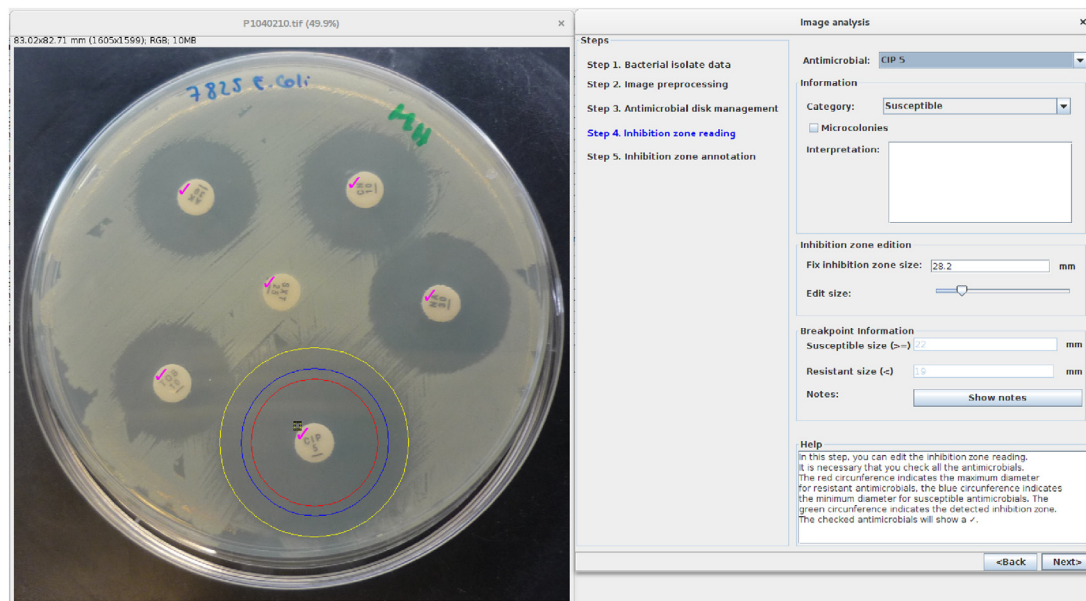


Fig. 5. Inhibition zone reading in AntibioqramJ. *Left.* Plate-image, the red circle indicates the limit of the resistant value, the blue circle indicates the limit of the susceptibility value, and the yellow circle the detected inhibition zone. *Right.* Plate analyser wizard showing (1) information about the selected antimicrobial (category, whether it has microcolonies (in the inhibition zone), and interpretation given by the user), (2) options to edit the inhibition zone, (3) breakpoint information provided by the standard. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Step 4. Inhibition zone reading. AntibioqramJ automatically determines and measures the inhibition zones of the plate-images, and categorises them based on the inhibition zone diameters and the values provided by the standard's breakpoints. The available categories are *Susceptible*, *Intermediate*, *Resistant*, and *Not Available* – the last option indicates that the standard does not provide a breakpoint for that antimicrobial. The user can manually correct the detected inhibition zones by using a slider or by manually introducing a diameter size. When an inhibition zone is modified, its category is automatically recomputed. In this step, the user can visualise the susceptibility limits and values: a red circle in the image indicates the limit of the resistant value, a blue circle indicates the limit of the susceptibility value, and a yellow circle the detected inhibition zone (see Fig. 5). This makes easier the categorisation of each inhibition zone, since the user can visualise it at a glance. Moreover, in this step, the user can provide an interpretative reading of the antibiogram as a complement of the susceptibility test result.

Step 5. Inhibition zone annotation. As a final step in the plate analyser wizard, the user can annotate the image including information like the category and diameter of each inhibition zone, and customise the information using different colours, see Fig. 6.

Finishing the plate analysis. Once the user has finished the analysis of a plate-image (i.e. the five aforementioned steps have been completed), a new plate is stored in the AntibioqramJ database and added to an antibiogram of the active experiment in the AntibioqramJ interface (see Figs. 1–3). The analysed plate can be added to an antibiogram of the experiment in two different ways. If the plate was inoculated with the same microorganism of one of the antibiograms already present in the experiment, the plate is incorporated to such an antibiogram; otherwise, a new antibiogram containing the plate is created and added to the experiment. In both cases, the process is conducted automatically by AntibioqramJ. Finally, if required, an alert system proposes additional phenotypic and molecular tests to detect specific resistances or resistance mechanisms according to the EUCAST guidelines (the default standard) [18].

In the main window of AntibioqramJ, if the active experiment is selected, a summary of the information of all the antibiograms of such an experiment is shown (see Fig. 1) – for each antibiogram of the experiment and for each antimicrobial of such antibiogram, the AntibioqramJ interface shows the name of the antimicrobial, the code, the category, the diameter, the standard employed to determine the susceptibility, and additional information provided by the researcher (e.g. interpretation, inner microcolonies and so on). If one of the antibiograms of the experiment is selected (see Fig. 2) only the information associated with such an antibiogram is shown in a new tab. Finally, if a plate of one of the antibiograms of the active experiment is selected, its associated tab is displayed showing the information of the plate, the antimicrobials employed in such a plate and its corresponding data, and the associated image (with or without the annotations), see Fig. 3.

Hence, from the main window of AntibioqramJ, the user can consult the readings of the antibiograms associated with each experiment. Moreover, AntibioqramJ allows the user to query the system.

2.3. Searching inside antibiogramJ

The main functionality of AntibioqramJ is the determination, measurement and categorisation of inhibition zones of plate-images. In addition, AntibioqramJ supplies the functionality to conduct different searches in the database that is created with the analysis of those images.

Search by antimicrobial. AntibioqramJ can search the bacterial isolates of the antibiograms stored in the database which are resistant, susceptible or intermediate to a concrete antimicrobial, see Fig. 7. The results can be filtered by microbial group and also by species. Moreover, the obtained results can be exported to an Excel file. Examples of queries that can be carried out using this search mechanism are: which strains of the database are resistant to Cefotaxime?, or which *Escherichia coli* strains are susceptible to Ampicillin?

This feature can be used as an epidemiological tool to determine the prevalence of resistance of a specific bacteria or bacte-

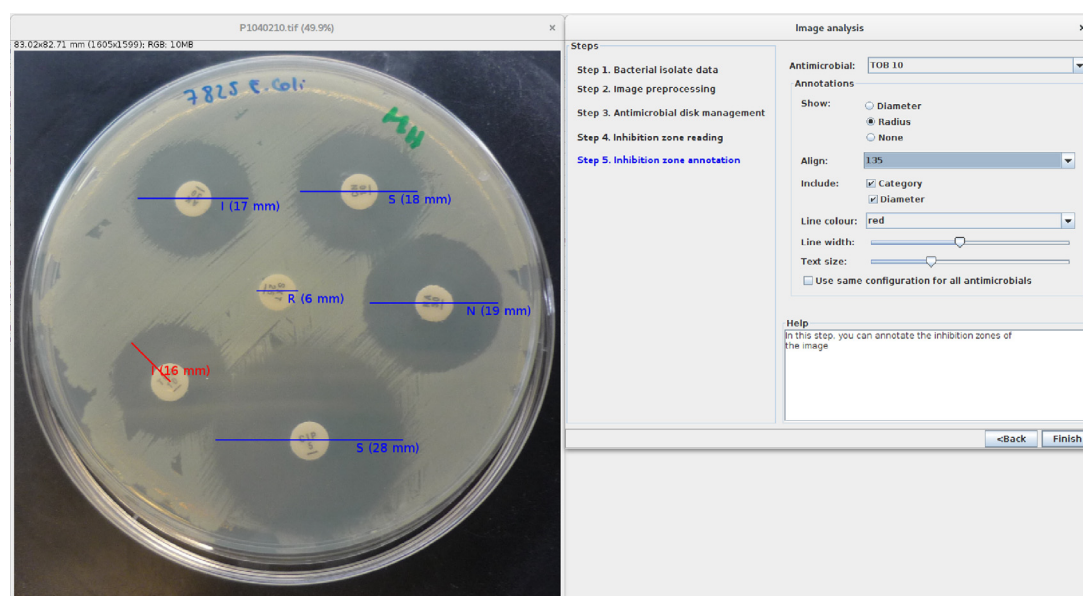


Fig. 6. Inhibition zone annotation in AntibioGramJ. *Left.* Plate-image annotated with different information. *Right.* Plate analyser wizard showing the different options to annotate the image.

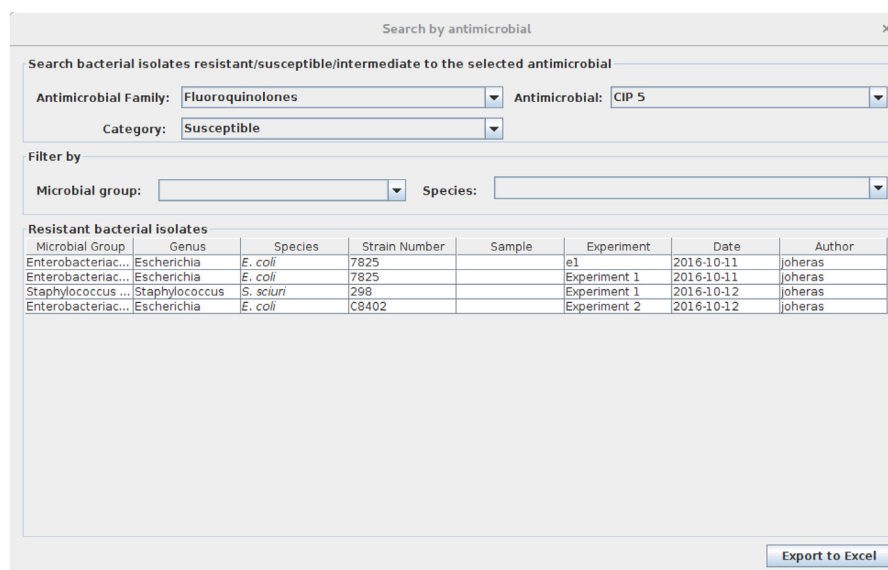


Fig. 7. Search by antimicrobial. In this window, all the bacterial isolates susceptible to antimicrobial CIP 5 are shown.

rial group against one or more antimicrobial agents. The determination of the prevalence of a particular mechanism of resistance (extended-spectrum beta-lactamase production, methicillin-resistance and so on) needs additional analyses as a complement of the susceptibility test results – the additional analyses are recommended based on specific phenotypic criteria established in the EUCAST guidelines by means of the alert system included in AntibioGramJ.

Search by bacterial isolates. The user of AntibioGramJ can select several bacterial isolates and ask the system for which antimicrobials the selected strains are either resistant or intermediate (see Fig. 8). As in the search by antimicrobial, the results can be exported to an Excel file. An example of what the user can ask using this feature is: “show me the antimicrobials for which the strains C2088, C3560 and C239S are resistant”.

Search by antibiograms. AntibioGramJ also allows the user to create new experiments from previously analysed antibiograms (see Fig. 9). The user can search antibiograms by any of their fields stored in the database, and filter the search using a “from” and a “to” date. Examples of the use of this feature are: “show me a list of all the *E. coli* antibiograms that I have tested” or “show me a list with all the antibiograms of Enterobacteriaceae from the 1st of January of 2017”.

3. Results

In this section, we compare AntibioGramJ’s automatic procedure to categorise inhibition zones with the results obtained manually by an expert microbiologist. For this study, we employed a battery of 86 plates-images corresponding to 27 enterobacterial, enterococcal, staphylococcal, pseudomonal and acinetobacterial clinical

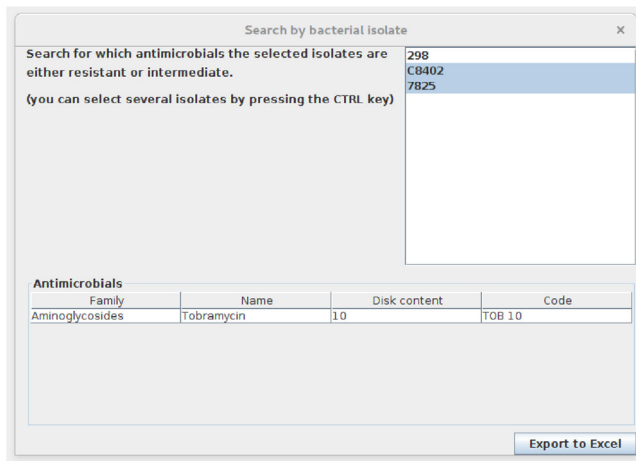


Fig. 8. Search by bacterial strain. In this window, it is shown for which antimicrobials, the strains C8402 and 7825 are resistant.

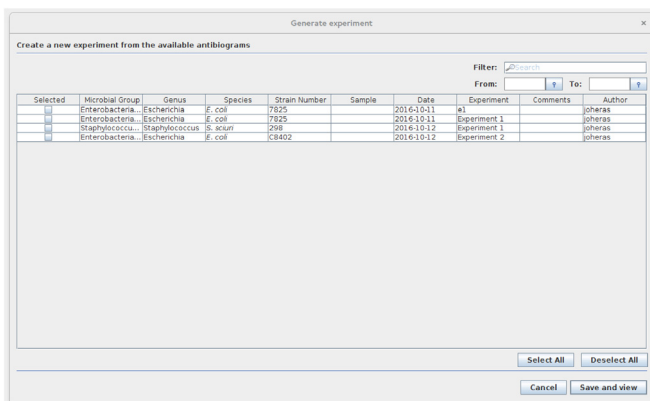


Fig. 9. Search by antibiograms.

isolates prepared on Mueller Hinton (MH) agar plates. A total of 29 different antimicrobial agents were used.

From the 86 plates-images, 43 images were captured with a digital camera with 8.1 MP, 3× (33–100 mm) optical zoom, and sensitivity to ISO 1600; and 43 images using a mobile phone with a camera with 13 MP and autofocus. The 86 plate-images were manually classified as good-quality images (55 images) and bad-quality images (31 images) depending on factors such as lightning conditions and intensity of inhibition zones. A total of 720 inhibition zones were analysed.

The EUCAST breakpoints [6] were used as susceptibility thresholds, allowing the classification of the isolates into three categories for each antimicrobial tested: *S* (sensible), *I* (intermediate), and *R* (resistant). The results obtained with AntibioGramJ were compared to the manual readings and expressed in terms of agreement and disagreement. Following the terminology employed in [19,20], identical characterization in both methods was defined as agreement; categorisation of *I* with one method and *R* or *S* with another method was defined as *minor disagreement*; categorisation of *S* in the manual reading and *R* in AntibioGramJ was defined as *major disagreement*; and categorisation of *R* in the manual reading and *S* in AntibioGramJ was defined as *very major disagreement*. The kappa index, which is commonly employed for comparing inter-rater agreement, was calculated using SPSS (IBM Corp.; Armonk, NY, USA).

The agreements and disagreements considering different criteria are shown in Table 1 – contingency tables are available in Appendix A. An overall agreement of 87% was observed and the

resulting kappa index was 0.768. Splitting the dataset of images into camera images and mobile images, the agreement percentages were 89% and 85% respectively, and the kappa value indexes were 0.8 and 0.736. If we only consider good-quality images, an agreement of 91% was observed with kappa index value of 0.844. In the case of bad-quality images, an agreement of 82% was observed with kappa index value of 0.663. Table 1 also contains the results split into families, and the agreements range from 71% to 93%.

As we have previously mentioned, the results presented in this section have been obtained applying AntibioGramJ fully automatically, that is, without any user interaction. However, it is worth remarking that AntibioGramJ is a semi-automatic system that allows the user to easily spot whether the automatic reading was correct, and otherwise modify it by just moving a slider. Therefore, with little effort, the users of AntibioGramJ can obtain correct readings in images acquired with any camera device.

4. Discussion

There are two kinds of automated or semi-automated inhibition zone readers: integrated instruments that combine hardware and software; and software tools that work with plate-images captured with a camera device.

For the first kind of inhibition zone readers, a variety of instruments have been introduced over the years to automate the measurement of inhibition zones in order to determine antimicrobial susceptibility [11,21–23]. Nowadays, these systems usually consists of three components: a holding area for inoculated plates, a robotic handling mechanism for the plates, and an image capture station. These instruments are typically combined with a middleware system that resides between the image capture system and the laboratory information system. Some of those instruments are: BIOMIC V3 (Giles Scientific; Santa Barbara, California), VITEK 2 (Biomérieux, USA), ADAGIO (BIO-RAD, Marne La Coquette, France), Sirscan 2000 (i2a, Montpellier, France), BD Kiestra (Drachten, Netherlands), Wasplap (Murrieta, California), bioMérieux (Marcy-l'Étoile, France) and Maestro (i2a, Montpellier, France).

The advantages of automation provided by those systems include a higher degree of standardisation resulting in an increased accuracy, improved data management with a concomitant reduction in transcription errors, earlier availability of results, and decrease exposure to cultivated pathogens [24]. Moreover, archived images of culture plates enable more thorough quality review and the development of real-world training sets, increase staff productivity, and facilitate comparison and sharing of cultures from multiple sites and at multiple time points during incubation [11]. In addition, the library of images may also be used as an educational tool [23].

Unfortunately, as indicated by the review published in 2008 [22], most integrated systems were too expensive for many laboratories, and this has not changed in the past years [11,23]. Another common challenge for integrating these systems in the laboratory is the change in the laboratory design and workflow, together with the training associated with any new technology.

In the second kind of inhibition zone readers, the aforementioned challenges are tackled by using an affordable camera device (e.g. a digital camera, a tablet or a mobile phone), and a software tool for automating zone inhibition measurements. This solution, while keeping most of the advantages of the integrated systems from the first approach, reduces the costs and changes in the laboratory design and workflow. However, there are only a few standalone software tools with this aim – the majority of software tools are part of embedded systems. In addition, the automation-degree is lower in this approach because images are captured in

Table 1
Agreements and disagreements for susceptibility categorisations.

	N	% Agreement	% Disagreement			Kappa index
			% Minor	% Major	% Very Major	
All	720	87	6	5	2	0.768
Camera	360	89	6	4	1	0.800
Mobile	360	85	6	6	3	0.736
Good-quality	386	91	2	3	3	0.844
Bad-quality	334	82	1	7	0	0.663
<i>Acinetobacter</i> spp.	130	93	0	0	7	0.551
Camera	65	100	0	0	0	1
Mobile	65	86	0	0	14	0.371
<i>Enterobacteriaceae</i>	138	93	4	3	0	0.872
Camera	69	94	3	3	0	0.886
Mobile	69	93	4	3	0	0.859
<i>Enterococcus</i> spp.	14	71	0	0	29	–
Camera	7	100	0	0	0	–
Mobile	7	43	0	0	57	–
<i>Pseudomonas</i> spp.	104	89	4	3	4	0.799
Camera	52	88	4	4	4	0.782
Mobile	52	90	4	2	4	0.816
<i>Staphylococcus</i> spp.	334	82	1	7	0	0.663
Camera	167	82	1	7	1	0.660
Mobile	167	82	1	8	0	0.667

an uncontrolled environment; and, hence, more user-interaction might be needed to process the images.

After a thorough search, we have found the following standalone tools for antibiogram analysis. A procedure to analyse plate-images using ImageJ was presented in [25] (<http://vlab.amrita.edu>). This procedure is completely manual and the final results are the diameters of the inhibition zones. ImageJ is also internally employed by diskImageR [26], an R package that determines the degree of drug susceptibility (the radius of inhibition), the subpopulation growth (fraction of growth within the zone of inhibition) and the rate of change in growth from no drug to inhibitory drug concentrations. These two systems lack some instrumental features like the determination of antimicrobial-disk codes, the availability of breakpoints to determine the category of the antimicrobial phenotype, or a database to store previously analysed plate-images. A more complete system, implemented in MATLAB, was presented by Costa et al. in [20]. This system is able to determine antimicrobial codes and inhibition zones, and from that information, it determines the susceptibility categorisation using the CLSI breakpoints. However, this system has some limitations: the antimicrobial codes that can be determined are limited to 12 antimicrobials, it assumes that a disk dispenser is employed (hence, the number of disks and their positions are fixed), it does not have an interface, and the program is not freely-available.

AntibiogramJ also follows the second approach and solves the limitations of the other software tools. First of all, AntibigramJ tries to reduce user-interaction as much as possible, and whenever such an interaction is necessary, it guides the user by means of tooltips, autocomplete fields, wizards and metaphors. In comparison with the system presented by Costa et al. in [20], AntibigramJ has several advantages: the number of antimicrobials that can be determined is not limited thanks to the learning system presented in Section 2.2, it does not assume the use of a disk dispenser, it has a simple-to-use interface, and it is open-source and free. In addition, AntibigramJ includes useful features such as an integrated database, functionality to query the system and export the results to Excel files, the possibility of loading and working with different standards like EUCAST and CLSI, the functionality to import/export experiments, and the visualisation of breakpoints directly on the images to facilitate the interpretation of antibiograms.

A comparison of the features available in the software tools for antibiogram analysis (i.e. the ImageJ procedure, diskImageR,

the software presented in [20], and AntibigramJ) is provided in Table 2. We consider three categories: general features, antibiogram reading features, and user-experience features. In the “general features” category, AntibigramJ is the only system that is open-source, independent of the operating system, depends on software (Java) that is available in most computers, and can work with images captured with any camera device. In the category of “antibiogram reading features”, AntibigramJ and the software presented by Costa et al. share some features such as the automatic determination of antimicrobial disks and the automatic identification of inhibition zones, but there are two aspects where AntibigramJ surpasses the tool of Costa et al.: the chance of post-editing the results obtained automatically by the software – as we have explained in the Results section, neither AntibigramJ nor the tool of by Costa et al. obtain perfect readings; hence, a post-edition is necessary to obtain the correct results – and the support of several breakpoints standards – not all the laboratories use the same breakpoints standards, and updates of those standards are usually required. Finally, AntibigramJ provides a better user-experience than the rest of the tools, since it provides several features, that in spite of not being necessary for reading antibiograms, help researchers in their work.

We finish this section by comparing the accuracy of AntibigramJ with the accuracy reported in the literature for other inhibition zone readers. As we have explained in the Results section, the users of AntibigramJ can obtain the correct results for images captured with any camera device thanks to the post-processing of the automatically detected inhibition zones. Such an accuracy has not been obtained by fully-automatic systems even when they use special-purpose devices to capture the plate-images. The fully-automated software presented by Costa et al. [20] has an agreement of 88% using a special-purpose camera device and imposing several restrictions (e.g. the use of a disk dispenser or a fixed number of antimicrobial disks per Petri dish). In the case of integrated systems with a fully automated analysis, the category agreements reported in the literature are similar to the ones obtained by AntibigramJ: Aura image system, 82.4% [22]; Osiris, 72.6% [27]; and ADAGIO, 81.4% [28]. Some integrated systems include, as AntibigramJ, a feature to manually adjust the automatically detected zone diameters, obtaining almost perfect accuracies: ADAGIO, 94.8% [28], Biomic Video Reader, 95% [29]; and, Sirscan, 97.8% [9].

Table 2
Features available in the software tools for antibiogram analysis.

	ImageJ procedure	diskImageR	Costa et al.	AntibiogramJ
Open-source	✓	✓		✓
Operating system	Independent	Independent	Independent	Independent
Dependencies	ImageJ	R	MATLAB	Java
Works with images captured with any camera device	Yes	Yes	No	Yes
Image preprocessing	✓		✓	✓
Determination of antimicrobial-disk codes			✓	✓
Identification algorithm	Manual	Automatic	Automatic	Automatic
Post-edition				✓
Visualisation of breakpoints				✓
CLSI support			✓	✓
EUCAST support				✓
Other breakpoints support				✓
Devoted GUI			✓	✓
Database support				✓
Image annotation				✓
Reports				✓
Export to Excel				✓
Import/Export				✓
Searching				✓

Therefore, AntibiogramJ automatically obtains comparable results to those obtained automatically by integrated systems but without requiring any investment in new equipment, and working with images captured with any camera device. Additionally, the visualisation and post-processing features of AntibiogramJ allow researchers to easily fix automatically-obtained incorrect results.

5. Conclusions

In this paper, we have presented AntibiogramJ, a Java application designed for determining, measuring and categorising zone of growth inhibition from plate-images captured with any camera device, including digital cameras, smartphones and tablets. AntibiogramJ is a user-friendly, platform-independent, open-source, and free-tool. Up to the best of our knowledge, AntibiogramJ is the most complete software tool for antibiogram analysis without requiring any particular hardware system. Besides, the agreement percentage obtained automatically by AntibiogramJ when compared to manual measurements is similar to those reported in the literature for automated inhibition zone readers. In addition, thanks to the features of AntibiogramJ, the researcher can easily detect when the automatic reading has failed and fix it to obtain the correct results.

As further work, some improvements could be introduced in AntibiogramJ to deal with some aspects that, currently, must be handled manually by the researcher. First of all, the algorithms implemented in AntibiogramJ are optimised to work with images of antibiograms that were performed on Mueller Hinton (MH) agar plates; hence, when working with blood-containing MH agar plates, the user might need to correct more frequently the inhibition zones detected automatically by AntibiogramJ – the rest of AntibiogramJ's functionality works equally well for both MH and blood-containing MH agar plates. Moreover, it would be interesting to include a feature to detect synergies in plate-images; since currently, this information must be annotated manually by the user. Furthermore, we envision two additional tasks: a client-server architecture and a mobile application for tablets. The former task will consist in changing the current local architecture of AntibiogramJ with a client-server architecture that allows the

users of this tool to share their experiments across the world. Currently, this can be achieved in AntibiogramJ by using the import/export functionality, but a client-server architecture will facilitate the comparison of cultures from multiple sites. The latter task refers to the creation of a mobile application with the same functionality as AntibiogramJ. It is our belief that, due to the fact that most tablets incorporate a camera, a mobile application for that kind of device will fasten the analysis of antibiograms since plate-images would be analysed directly in the same device used for capturing the images.

Availability and requirements

- Project name: AntibiogramJ.
- Project home page: <https://sourceforge.net/projects/antibiogramj/>.
- Operating system(s): Platform independent.
- Programming language: Java.
- Other requirements: Java 8.
- License: GNU GPL v3.
- Any restrictions to use by non-academics: restrictions specified by GNU GPL v3.

AntibiogramJ does not require installation. To run AntibiogramJ, the user should download AntibiogramJ from the project home page, unzip the downloaded file, and run the program *antibiogramjv1*.jar*. The only requirement to run AntibiogramJ is the installation of Java.

Several videos explaining how to use AntibiogramJ are available in the Wiki page of the project home page.

Competing interests

The authors declare that they have no competing interests.

Acknowledgement

This work was partially supported by Ministerio de Economía y Competitividad [MTM2014-54151-P].

Appendix A. Contingency tables

Table A1

Contingency tables all images.

		Antibiogram]														
		All images			Camera images			Mobile images			Good-Quality			Bad-Quality		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Manual	S	325	33	36	165	17	16	160	16	20	149	8	11	176	25	25
	I	0	25	1	0	12	0	0	13	1	0	18	0	0	7	1
	R	14	9	277	3	4	143	11	5	134	13	1	186	1	8	91

Table A2

Contingency tables Acinetobacter.

		Antibiogram]								
		All images			Camera images			Mobile images		
		S	I	R	S	I	R	S	I	R
Manual	S	0	0	0	0	0	0	0	0	0
	I	0	6	0	0	3	0	0	3	0
	R	9	0	115	0	0	62	9	0	53

Table A3

Contingency tables Enterobacteriaceae.

		Antibiogram]								
		All images			Camera images			Mobile images		
		S	I	R	S	I	R	S	I	R
Manual	S	80	4	4	40	2	2	40	2	2
	I	0	4	0	0	2	0	0	2	0
	R	0	1	45	0	0	23	0	1	22

Table A4

Contingency tables Enterococcus.

		Antibiogram]								
		All images			Camera images			Mobile images		
		S	I	R	S	I	R	S	I	R
Manual	S	10	0	4	7	0	0	3	0	4
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0

Table A5

Contingency tables Pseudomonas.

		Antibiogram]								
		All images			Camera images			Mobile images		
		S	I	R	S	I	R	S	I	R
Manual	S	59	4	3	29	2	2	30	2	1
	I	0	8	0	0	4	0	0	4	0
	R	4	0	26	2	0	13	2	0	13

Table A6

Contingency tables Staphylococcus.

		Antibiogram]								
		All images			Camera images			Mobile images		
		S	I	R	S	I	R	S	I	R
Manual	S	176	25	25	89	13	12	87	12	13
	I	0	7	1	0	3	0	0	4	1
	R	1	8	91	1	4	45	0	4	46

Supplementary material

Supplementary material associated with this article can be found, in the online version, at [10.1016/j.combustflame.2015.09.001](https://doi.org/10.1016/j.combustflame.2015.09.001).

References

- [1] S.G. Jenkins, A.N. Schuetz, Current concepts in laboratory testing to guide antimicrobial therapy, *Mayo Clin. Proc.* 87 (3) (2012) 290–675.
- [2] E. Matuschek, D.F. Brown, G. Kahlmeter, Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories, *Clin. Microbiol. Infect.* 20 (4) (2014) Q255–66.
- [3] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck, Antibiotic susceptibility testing by a standardized single disk method, *Am. J. Clin. Pathol.* 45 (1966) 493–496.
- [4] L.B. Reller, M. Weinstein, J.H. Jorgensen, M.J. Ferraro, Antimicrobial susceptibility testing: a review of general principles and contemporary practices, *Clin. Infect. Dis.* 49 (11) (2009) 1749–1755.
- [5] Clinical, L.S. Institute, Performance standards for antimicrobial susceptibility testing, CLSI, 26, Clinical and Laboratory Standards Institute, Wayne, PA, 2016. Supplement M100S.
- [6] E.Co. A. S. Testing, Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016.
- [7] R. Cantón, Interpretive reading of the antibiogram: intellectual exercise or clinical need? *Enfermedades Infecciosas y Microbiología Clínica* 20 (4) (2002) 176–185.
- [8] D.M. Livermore, T.G. Winstanley, K.P. Shannon, Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes, *J. Antimicrob. Chemother.* 48 (2001) 87–102.
- [9] M. Hombach, R. Zbinden, E.C. Böttger, Standardisation of disk diffusion results for antibiotic susceptibility testing using the sirscan automated zone reader, *BMC Microbiol.* 13 (255) (2013).
- [10] D. Felmingham, D.F.J. Brown, Instrumentation in antimicrobial susceptibility testing, *J. Antimicrob. Chemother.* 48 (S1) (2001) 81–85.
- [11] D.D. Rhoads, S.M. Novak, L. Pantanowitz, A review of the current state of digital plate reading of cultures in clinical microbiology, *J. Pathol. Inform.* 6 (23) (2015).
- [12] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH image to imageJ, *Nat. Methods* 9 (2012) 671–675.
- [13] A. Kaehler, G. Bradski, *Learning openCV 3*, O'Reilly Media, 2015.
- [14] Oracle, *JavaDB Reference Manual*, Technical Report, 2014.
- [15] B. Evjen, et al., *Professional XML*, Wiley Publishing Inc, 2007.
- [16] H.K. Yuen, et al., Comparative study of hough transform methods for circle finding, *Image Vis. Comput.* 8 (1) (1990) 71–77.
- [17] E. Rublee, et al., ORB: an efficient alternative to SIFT or SURF, in: *Proceedings of International Conference on Computer Vision*, IEEE, 2011, pp. 2564–2571.
- [18] E.Co. A. S. Testing, EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, 2013. http://www.eucast.org/resistance_mechanisms/.
- [19] G. Hejblum, et al., Automated interpretation of disk diffusion antibiotic susceptibility tests with the radial profile analysis algorithm, *J. Clin. Microbiol.* 31 (1993) 2396–2401.
- [20] L.F.R. Costa, et al., Development of an automatic identification algorithm for antibiogram analysis, *Comput. Biol. Med.* 67 (2015) 104–115.
- [21] A. Croxatto, et al., Laboratory automation in clinical bacteriology: what system to choose? *Clin. Microbiol. Infect.* 22 (3) (2016) 217–235.
- [22] E.S. Lestari, et al., Comparison of the accuracy of disk diffusion zone diameters obtained by manual zone measurements to that by automated zone measurements to determine antimicrobial susceptibility, *J. Microbiol. Methods* 75 (2) (2008) 177–181.
- [23] A.B. Mochon, M.S. Cruz, Telemicrobiology: focusing on quality in an era of laboratory consolidation, *Clin. Microbiol. NewsL.* 38 (3) (2016) 19–24.
- [24] H.K. Geiss, U.E. Klar, Evaluation of the BIOMIC video reader system for routine use in the clinical microbiology laboratory, *Diagn. Microbiol. Infect. Dis.* 37 (3) (2000) 151–155.
- [25] vlab.amrita.edu, Quantification of bacterial colonies on an agar plate, 2012Last accessed on 24th November 2016.
- [26] A.C. Gerstein, et al., Diskimager: quantification of resistance and tolerance to antimicrobial drugs using disk diffusion assays, *Microbiology* (2016). DOI: 10.1099/mic.0.000295
- [27] M. Kolbert, F. Chegrani, P.M. Shah, Evaluation of the OSIRIS video reader as an automated measurement system for the agar disk diffusion technique, *Clin. Microbiol. Infect.* 10 (5) (2004) 416–420.
- [28] E.A. Idelevich, Evaluation of an automated system for reading and interpreting disk diffusion antimicrobial susceptibility testing of fastidious bacteria, *PLoS ONE* 11 (7) (2016) e0159183. DOI: 10.1371/journal.pone.0159183
- [29] E.K. Korgenski, J.A. Daly, Evaluation of the BIOMIC video reader system for determining interpretive categories of isolates on the basis of disk diffusion susceptibility results, *J. Clin. Microbiol.* 36 (1) (1998) 302–304.