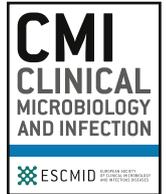




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## Original article

## Comparison of the Copan WASPLab incorporating the BioRad expert system against the SIRscan 2000 automatic for routine antimicrobial disc diffusion susceptibility testing

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## ABSTRACT

**Objectives:** This study investigated the agreement at the categorical level between the Copan WASPLab incorporating the BioRad expert system against the SIRscan 2000 automatic for antimicrobial disc diffusion susceptibility testing.

**Methods:** The 338 clinical strains (67 *Pseudomonas aeruginosa*, 19 methicillin-resistant *Staphylococcus aureus*, 75 methicillin-sensitive *S. aureus* and 177 *Enterobacteriales* isolates) analysed in this study were non-duplicate isolates obtained from consecutive clinical samples referred to the clinical bacteriology laboratory at Geneva University Hospitals between June and August 2019. For the WASPLab the inoculum suspension was prepared in strict accordance with the manufacturer's instruction (Copan WASP srl, Brescia, Italy) by adding 2 mL of the 0.5 McFarland primary suspension used for the SIRscan analysis into a sterile tube filled with 4 mL of sterile saline (1:3 dilution). The inoculum (2 × 30 µL loop/spreader) was spread over the entire surface of Mueller–Hinton agar plates according to the AST streaking pattern defined by Copan. The antibiotic discs were dispensed by the WASP and inoculated media were loaded on conveyors for transfer to the automatic incubators. The plates were incubated for 16 h, and several digital images were acquired. Inhibition zone diameters were automatically read by the WASPLab and were adjusted manually whenever necessary. For the SIRscan 2000 automatic, the antimicrobial disc diffusion susceptibility testing was performed according to the EUCAST guidelines. The gradient strip method was used to resolve discrepancies.

**Results:** The overall categorical agreement between the compared methods reached 99.1% (797/804; 95% CI 98.2%–99.6%), 99.5% (1029/1034; 95% CI 98.9%–99.8%), and 98.8% (2798/2832; 95% CI 98.3%–99.1%) for *P. aeruginosa*, *S. aureus* and the *Enterobacteriales*, respectively.

**Conclusions:** WASPLab incorporating the BioRad expert system provides a fully automated solution for antimicrobial disc diffusion susceptibility testing with equal or better accuracy than other available phenotypic methods. **A. Cherkaoui, Clin Microbiol Infect 2019;•:1**

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## Introduction

Phenotypic antimicrobial susceptibility testing (AST) entails an agreement on breakpoints and a rigorous standardization of

methods and materials. Phenotypic AST methods involve culturing a sample to obtain a pure culture, permitting its testing, under defined bacterial and drug concentrations, to determine which antimicrobial agents kill or inhibit the bacterial growth. The disc diffusion method is one of the oldest approaches and remains one of the most widely used methods in routine clinical microbiology laboratories. The method is versatile in that it is appropriate for determining AST for the most bacterial pathogens, including less

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common fastidious bacteria. Almost all antimicrobial agents can be tested without special equipment, ensuring greatest flexibility and cost-effectiveness. The disc diffusion method assesses antimicrobial susceptibility based on the growth response of bacteria exposed to discs impregnated with antibiotics. This method is based on the original work of Bauer et al., where the diffusion of an antibiotic through the agar establishes a reproducible concentration gradient [1]. The growth of the tested bacteria inoculated on a specific agar medium at a defined concentration (CFU/mL) reproducibly leaves an inhibition zone around the disc where the antibiotic concentration is sufficient to inhibit growth. The inhibition zone diameter defining susceptibility or resistance is determined for each drug/bug combination after a large validation based on correlation between the MIC and the inhibition zone size, pharmacokinetic parameters, pharmacodynamics indices of the antimicrobial agent, and the results of clinical trials. At an international level, the wide variation in breakpoints for AST has long been recognized as an issue. As different breakpoints lead to different reports of susceptibility for some isolates, they make the comparison of resistance rates across surveillance studies difficult. The European Committee for Antimicrobial Susceptibility Testing (EUCAST) was initiated to harmonize the clinical breakpoints across Europe. The major disadvantages of the disc diffusion method include lack of interpretive criteria for some antibiotics, and the inability to provide precise data regarding the level of an organism's resistance that can be quantified by other MIC methods. Additionally, plating, placing of discs and interpreting inhibition zones can be time-consuming when performed manually.

The purpose of this study was to investigate the agreement between the WASPLab incorporating the BioRad expert system with the SIRscan 2000 automatic on a panel of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and different species of *Enterobacteriales*, routinely isolated at Geneva University Hospitals.

## Methods

### Setting

The present study was carried out in the bacteriology laboratory of Geneva University Hospitals, a Swiss tertiary care centre with 1920 beds. We analyse about 165 000 clinical samples per year. Daily, we perform more than 120 AST panels, which requires a full-time-equivalent technician. Our current hours of operation extend from 07.30 to 22.00 (7/7).

### Bacterial strains

All the 338 strains (67 *P. aeruginosa*, 19 methicillin-resistant *S. aureus*, 75 methicillin-sensitive *S. aureus*, and 177 *Enterobacteriales*-isolates) analysed in this study were non-duplicate isolates obtained from consecutive clinical samples referred to the clinical bacteriology laboratory at Geneva University Hospitals between June and August 2019. Table 1 depicts the 14 different species of *Enterobacteriales* included in this study. The identification of the strains was performed by matrix-assisted desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany) according to the manufacturer's instructions.

### Antimicrobial disc diffusion susceptibility testing

#### Antibiotics tested

The AST panel for *P. aeruginosa* consisted of 12 different antibiotics (piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin and levofloxacin). The AST panel

**Table 1**  
*Enterobacteriales* species included in this study

Species	Number of clinical strains
<i>Escherichia coli</i>	37
<i>Escherichia coli</i> ESBL	46
<i>Klebsiella pneumoniae</i>	24
<i>Klebsiella pneumoniae</i> ESBL	18
<i>Proteus mirabilis</i>	8
<i>Enterobacter cloacae</i> complex	16
<i>Klebsiella oxytoca</i>	4
<i>Klebsiella aerogenes</i>	3
<i>Hafnia alvei</i>	1
<i>Morganella morganii</i>	5
<i>Citrobacter freundii</i>	4
<i>Serratia marcescens</i>	3
<i>Providencia rettgeri</i>	1
<i>Citrobacter koseri</i>	4
<i>Citrobacter braakii</i>	1
<i>Salmonella</i>	2
Total	177

for *S. aureus* consisted of 11 different antibiotics (cefoxitin, gentamicin, erythromycin, clindamycin, fusidic acid, co-trimoxazole, rifampicin, tigecycline, tetracycline, ciprofloxacin and linezolid). The AST panel for *Enterobacteriales* consisted of 16 different antibiotics (ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefuroxime, ceftazidime, ceftriaxone, cefepime, imipenem, meropenem, ertapenem, aztreonam, amikacin, gentamicin, norfloxacin, ciprofloxacin and co-trimoxazole).

### SIRscan 2000 automatic

Disc diffusion was performed according to the EUCAST guidelines. The inoculum suspension was prepared by picking several colonies from overnight growth on agar plates with a cotton swab and suspending the colonies in sterile saline (0.85% NaCl weight/volume in water) to a density of 0.5 McFarland. The inoculum was spread manually over the entire surface of the Mueller–Hinton agar plates (square plates were obtained from bioMérieux (Marcy l'Étoile, France) and round plates from BioRad (Marnes-la-Coquette, France)) by swabbing in three directions, and incubated in a humid atmosphere at  $35 \pm 1^\circ\text{C}$ , for 16 h. Inhibition zone diameters were automatically read by the SIRscan and were adjusted manually whenever deemed necessary. Interpretation followed the EUCAST breakpoint tables, version 9.0 (2019). All the antibiotic discs and dispensers were from i2a (Montpellier, France).

### WASPLab incorporating the BioRad expert system

The inoculum suspension was prepared in strict accordance with the manufacturer's instruction (Copan WASP srl, Brescia, Italy) by adding 2 mL of the 0.5 McFarland primary suspension used for the SIRscan 2000 automatic analysis into a sterile tube filled with 4 mL of sterile saline (0.85% NaCl weight/volume in water). A final volume of 6 mL was obtained (1:3 dilutions of the 0.5 McFarland primary suspensions). The inoculums ( $2 \times 30 \mu\text{L}$  loop/spreader) was spread over the entire surface of the round Mueller–Hinton agar plates according to the AST streaking pattern defined by Copan. The antibiotic discs were dispensed by the WASP and inoculated media were loaded on conveyors for transfer between WASP and automated incubators (see Supplementary material, Fig. S1). Plates were incubated for 16 h on the WASPLab, and several high-resolution digital images were acquired under different light and exposure conditions according to the manufacturer's instructions. Inhibition zone diameters were automatically read by the WASPLab and the inhibition zone diameters were adjusted manually when deemed necessary. The AST interpretation was

performed by the BioRad expert system according to EUCAST breakpoint tables. All the antibiotic discs and dispensers were from BioRad.

The Copan AST module implemented in our laboratory allows the execution of all steps of the AST (streaking, distribution of the antibiotic discs, incubation, acquisition of high-resolution digital images, automatic reading of the inhibition zone diameters and AST interpretation according to EUCAST breakpoint tables), making it work as an independent system.

#### Internal quality controls

Fifteen independent biological replicates of *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as internal quality controls to assess the accuracy, reproducibility and repeatability of the WASPLab AST method.

#### Discordant results

The WASPLab incorporating the BioRad expert system results were compared to the SIRscan 2000 automatic results, the latter being routinely performed in our laboratory for about 15 years. When both methods agreed, we considered the susceptibility category as correct and no further determination was performed. When the methods gave discordant results, we performed the gradient strip method (Etest, bioMérieux) according to the manufacturer's instructions. The discordant results were scored as 'very major error' if reported susceptible by the WASPLab or SIRscan but intermediate or resistant by Etest and as 'major error' if intermediate or resistant by the WASPLab or SIRscan but susceptible by Etest. No further molecular characterization to assess resistance mechanisms were performed in this study.

#### Ethical approval

In accordance with the local ethics committee (Commission cantonale d'éthique de la recherche, <https://www.hug-ge.ch/ethique>), routine clinical laboratories of our institution may use biological sample leftovers for method development after irreversible anonymization of the data.

## Results

### *Pseudomonas aeruginosa*

No discordant results at categorical level were observed for piperacillin, piperacillin-tazobactam, ceftazidime, aztreonam, imipenem, meropenem and tobramycin between the two compared methods, as depicted in Supplementary material (Fig. S2), which shows the distribution of the inhibition zone diameters for these antibiotics as measured by WASPLab and SIRscan. One major error was observed for levofloxacin by the WASPLab. The SIRscan AST showed two very major errors for levofloxacin and one very major error for ciprofloxacin. In addition, three major errors were recorded for cefepime amikacin, and gentamicin (Table 2 and see Supplementary material, Fig. S3). For *P. aeruginosa* the overall categorical agreement between both methods reached 99.1% (797/804; 95% CI 98.21%–99.58%).

### *Staphylococcus aureus*

No discordant results at categorical level were observed for ceftazidime, gentamicin, erythromycin, fusidic acid, co-trimoxazole, rifampicin and tigecycline between the two compared methods (see Supplementary material, Fig. S4). One major error was

**Table 2**

Discordant results at categorical level observed for *Pseudomonas aeruginosa* strains

Antibiotic	Measured diameter (mm)		MIC (mg/L)
	SIRscan	WASPLab	Etest
Cefepime	16 (R)	21 (S)	6 (S)
Amikacin	15 (I)	20 (S)	1.5 (S)
Gentamicin	12 (R)	17 (S)	1.5 (S)
Ciprofloxacin	30 (S)	21 (R)	1 (R)
Levofloxacin	29 (S)	14 (R)	4 (R)
	28 (S)	12 (R)	0.38 (S)
	23 (S)	20 (R)	1.5 (R)

Abbreviations: R, resistant; S, susceptible; I, intermediate.

observed for ciprofloxacin by the SIRscan. Four major errors were observed by the WASPLab: two for linezolid, one for clindamycin and one for tetracycline (Table 3, and see Supplementary material, Fig. S5). For *S. aureus*, the overall categorical agreement between the two compared methods yielded to 99.5% (1029/1034; 95% CI 98.87%–99.79%).

### Enterobacterales

No discordant results at categorical level were observed for ampicillin; ceftazidime, ceftriaxone, aztreonam and norfloxacin (see Supplementary material, Fig. S6). Twenty-six major errors were observed on the WASPLab: one for piperacillin-tazobactam, one for imipenem, two for meropenem, one for ertapenem, one for co-trimoxazole, three for gentamicin, four for ciprofloxacin, six for cefturoxime, and seven for amikacin. Five very major errors and three major errors were observed on the SIRscan (Table 4, and see Supplementary material, Fig. S7). For *Enterobacterales*, the overall categorical agreement between the two compared methods was 98.8% (2798/2832; 95% CI 98.33%–99.14%). Despite the important number of discordant results between the WASPLab and the SIRscan, the categorical agreement was >95% for every antibiotic tested.

#### Internal quality controls

Figs. 1–3 depict the distribution of inhibition zone diameters on WASPLab using BioRad antibiotic discs for 15 independent biological replicates of *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922, respectively. For *P. aeruginosa* ATCC 27853, three out of 180 antibiotic discs tested have shown inhibition zone diameters smaller than the threshold defined by EUCAST. Similar observations were made for *E. coli* ATCC 25922. For *S. aureus* ATCC 29213, five out of 165 antibiotic discs tested have shown inhibition zone diameters smaller than the threshold defined by EUCAST. In contrast, for all the antibiotics tested on SIRscan using i2A antibiotic discs, the inhibition zone diameters for the 3 × 15 independent

**Table 3**

Discordant results at categorical level observed for *Staphylococcus aureus* strains

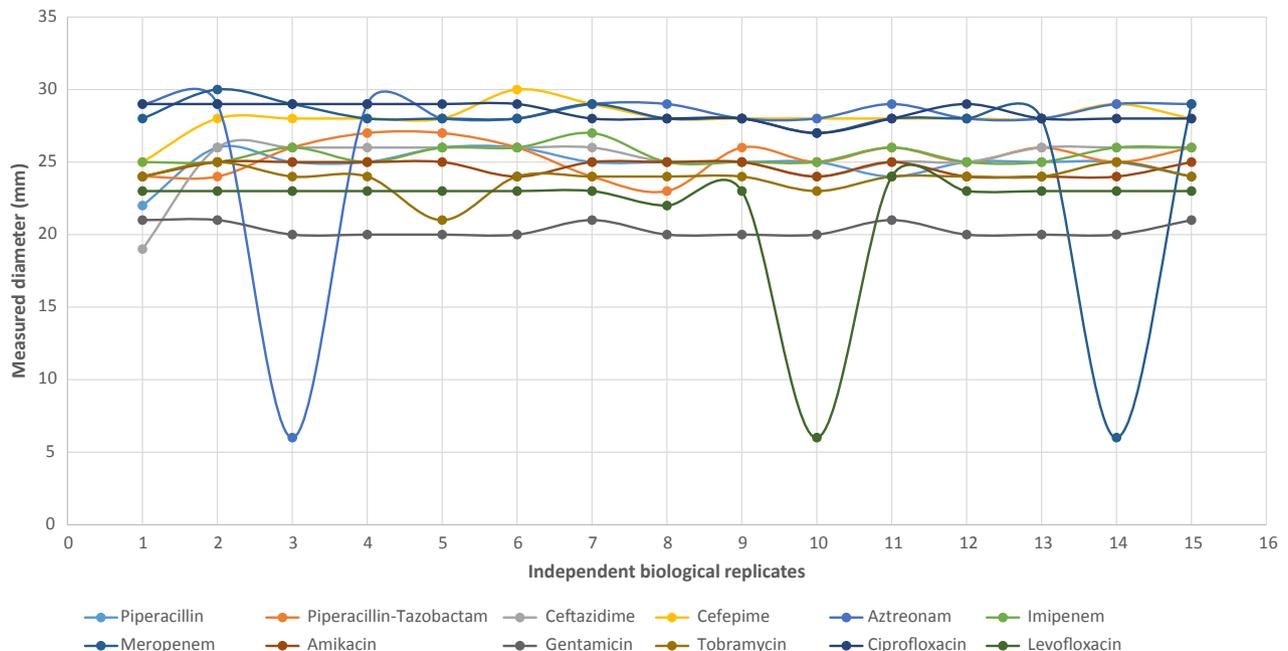
Antibiotic	Measured diameter (mm)		MIC (mg/L)
	SIRscan	WASPLab	Etest
Ciprofloxacin	23 (S)	19 (R)	0.19 (S)
Clindamycin	17 (R)	23 (S)	0.25 (S)
Tetracycline	37 (S)	15 (R)	0.064 (S)
Linezolid	26 (S)	6 (R)	1 (S)
	25 (S)	6 (R)	1.5 (S)

Abbreviations: R, resistant; S, susceptible.

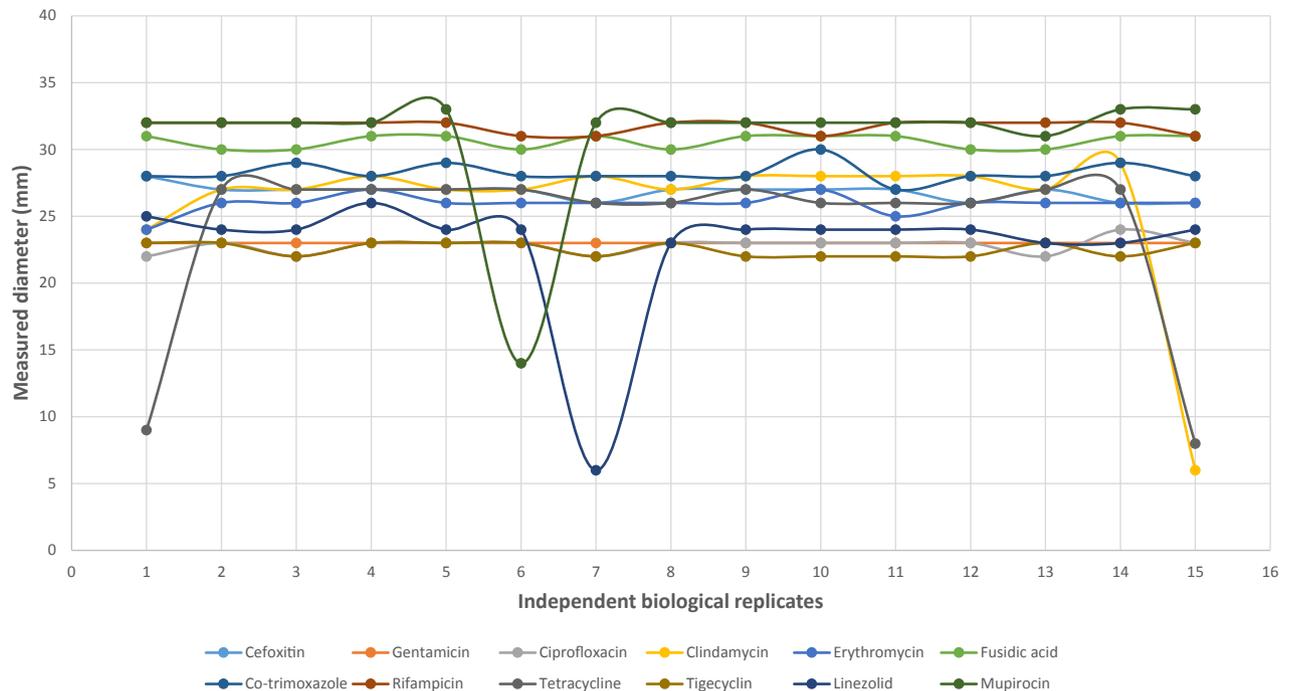
**Table 4**  
Discordant results at categorical level observed for *Enterobacterales* strains

Antibiotic	Species	Measured diameter (mm)		MIC (mg/L)	
		SIRscan	WASPLab	Etest	
Amoxicillin/clavulanate	<i>Klebsiella pneumoniae</i>	14 (R)	21 (S)	4 (S)	
Piperacillin/Tazobactam	<i>K. pneumoniae</i>	19 (I)	21 (S)	3 (S)	
	<i>Escherichia coli</i> ESBL	13 (R)	24 (S)	2 (S)	
	<i>E. coli</i> ESBL	25 (S)	14 (R)	256 (R)	
Cefuroxime	<i>E. coli</i> ESBL	26 (S)	6 (R)	0.75 (S)	
	<i>K. pneumoniae</i>	22 (S)	16 (R)	2 (S)	
	<i>Enterobacter cloacae</i>	23 (S)	14 (R)	6 (S)	
	<i>E. coli</i>	27 (S)	6 (R)	2 (S)	
	<i>K. pneumoniae</i>	26 (S)	6 (R)	3 (S)	
	<i>E. coli</i>	22 (S)	6 (R)	6 (S)	
	<i>Hafnia alvei</i>	30 (S)	16 (R)	2 (S)	
Cefepime	<i>K. pneumoniae</i> ESBL	27 (S)	22 (I)	1.5 (I)	
Imipenem	<i>E. coli</i> ESBL	29 (S)	18 (I)	0.25 (S)	
Meropenem	<i>E. coli</i> ESBL	31 (S)	6 (R)	0.094 (S)	
	<i>Enterobacter cloacae</i>	31(S)	19 (I)	0.25 (S)	
Ertapenem	<i>Klebsiella oxytoca</i>	35 (S)	6 (R)	0.003 (S)	
Amikacin	<i>E. coli</i> ESBL	23 (S)	14 (R)	48 (R)	
	<i>Proteus mirabilis</i>	24 (S)	14 (R)	1.5 (S)	
	<i>E. coli</i>	26 (S)	6 (R)	1.5 (S)	
	<i>Proteus mirabilis</i>	25 (S)	15 (I)	3 (S)	
	<i>E. coli</i> ESBL	23 (S)	14 (R)	6 (S)	
	<i>E. coli</i> ESBL	19 (S)	15 (I)	6 (S)	
	<i>Proteus mirabilis</i>	28 (S)	15 (I)	4 (S)	
	<i>Citrobacter freundii</i>	25 (S)	6 (R)	1.5 (S)	
	Gentamicin	<i>E. coli</i>	24 (S)	6 (R)	0.38 (S)
		<i>E. coli</i> ESBL	18 (S)	6 (R)	0.5 (S)
		<i>E. coli</i> ESBL	19 (S)	6 (R)	0.75 (S)
		<i>Proteus mirabilis</i>	27 (S)	23 (I)	0.25 (S)
	Ciprofloxacin	<i>E. coli</i> ESBL	28 (S)	23 (I)	0.19 (S)
<i>K. oxytoca</i>		36 (S)	19 (R)	0.012 (S)	
<i>Enterobacter cloacae</i>		29 (S)	6 (R)	0.023 (S)	
Co-trimoxazole		<i>Proteus mirabilis</i>	20 (S)	8 (R)	>32 (R)
		<i>Enterobacter cloacae</i>	28 (S)	6 (R)	0.094 (S)
	<i>K. pneumoniae</i> ESBL	19 (S)	7 (R)	12 (R)	

Abbreviations: ESBL, extended-spectrum  $\beta$ -lactamase; R, resistant; S, susceptible; I, intermediate.



**Fig. 1.** Distribution of inhibition zone diameters on WASPLab using BioRad antibiotic discs for different antibiotics tested for 15 independent biological replicates of *Pseudomonas aeruginosa* ATCC 27853.



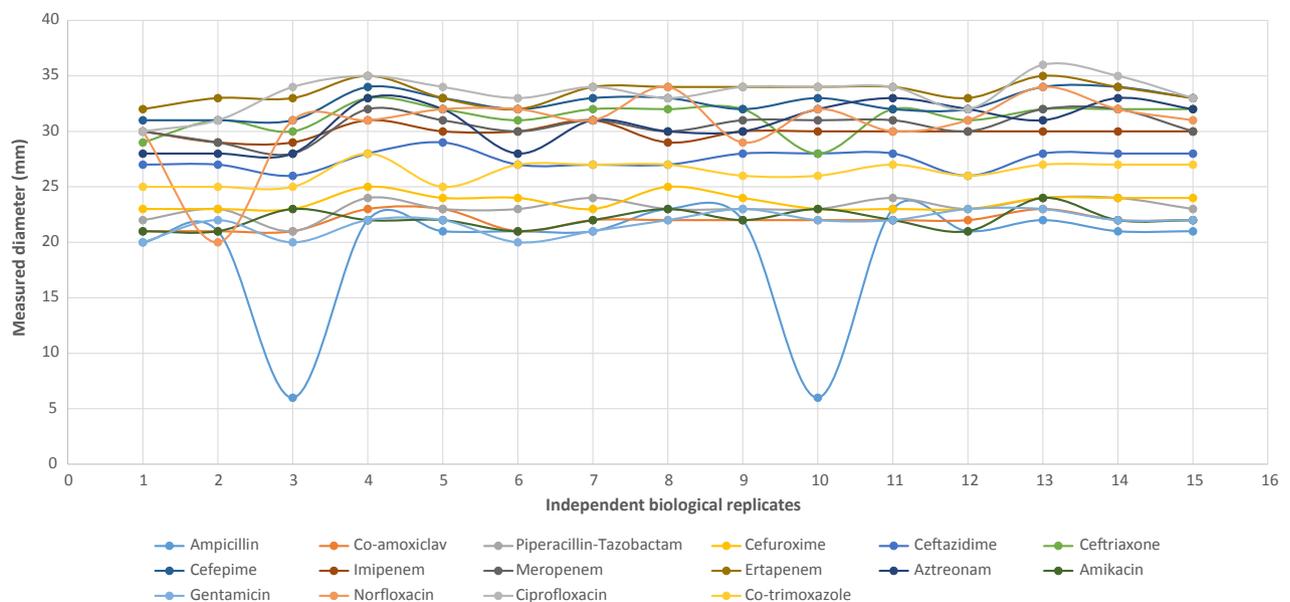
**Fig. 2.** Distribution of inhibition zone diameters on WASPLab using BioRad antibiotic discs for different antibiotics tested for 15 independent biological replicates of *Staphylococcus aureus* ATCC 29213.

biological replicates were always in the range defined by EUCAST (see Supplementary material, Figs S8, S9, S10).

## Discussion

The need for phenotypic susceptibility testing increases with escalating levels of antimicrobial resistance. In seriously ill individuals, the time elapsed to delivery of antimicrobial treatment is of vital importance [2]. Timely and reliable susceptibility

reports from the clinical laboratory facilitate antibiotic stewardship programmes by enabling de-escalation from broad-spectrum to targeted narrow-spectrum antibiotic treatment. Currently, phenotypic susceptibility testing remains necessary because there are many different mechanisms of resistance that challenge the generalization of genotype-based approaches [3–6]. Moreover, PCR-based approaches are able to identify only known resistance determinants, but cannot report on susceptibility.



**Fig. 3.** Distribution of inhibition zone diameters on WASPLab using BioRad antibiotic discs for different antibiotics tested for 15 independent biological replicates of *Escherichia coli* ATCC 25922.

Implementation of automated instrument systems permits specimen streaking, and transfer of the inoculated media to the incubators where cultures are assessed with high-resolution digital imaging at pre-defined times. This total automation has improved productivity, traceability and quality in routine clinical microbiology laboratories [7,8]. Furthermore, it has significantly reduced the turn-around time [8,9]. In order to assess the medical value of the WASPLab for antimicrobial disc diffusion testing, it is mandatory to document that the method is applicable to a wide diversity of clinically relevant organisms and to demonstrate that the modified protocol for inoculum preparation and streaking validated by Copan has a minimal impact on the quality of the results. The goal of this study was to use standardized data collection to assess the performance of the WASPLab AST under real routine laboratory conditions. The ultimate objective will be to operate the WASPLab as the first-line AST strategy; that is, as a method capable of performing most routine AST. This study compares the accuracy of the WASPLab incorporating the BioRad expert system against the SIRscan 2000 automatic, which represents the routine method used in our laboratory. Another objective was to assess whether the WASPLab, used for AST immediately after bacterial isolation, could reduce laboratory turn-around time without affecting accuracy. The overall categorical agreement between the compared methods yielded 99% (4624/4670; 95% CI 98.69%–99.26%). Thirty-one major errors were observed with the WASPLab against only seven for the SIRscan. Eight very major errors were also noticed for the SIRscan (Table 5). Despite the fact that no very major error was observed with the WASPLab, we were nevertheless surprised by the large number of major errors in *Enterobacteriales*. The basic assumptions were that the antibiotic discs were not correctly dosed or that the antibiotic discs were affected by the heat in the WASP area. The first hypothesis was confirmed by repeating the AST on the WASPLab for each discordant strain (i.e. showing 6-mm inhibition zone on WASPLab). All these strains were revealed to be clearly susceptible, as confirmed by the SIRscan and the Etest. Furthermore, the analysis of the internal quality controls with ATCC strains also confirmed that all major errors observed could be attributable to the manufacturing of antibiotic-impregnated discs. To avoid the effects of temperature in the WASP area, dispensers and antibiotic cartridges are always kept at 4°C and installed in the WASP only when performing the AST. These discordant results highlight the importance of running daily internal quality controls using ATCC strains, as recommended by EUCAST.

The following factors are critical to the accuracy and repeatability of the disc diffusion method: bacterial inoculum preparation, manual streaking of media plates, disc content, agar medium, nutritional requirements, incubation temperature and atmosphere, incubation time and subjectivity of inhibition zone reading [10–12]. The WASPLab performs the streaking of bacterial inoculum and dispenses antibiotic discs, the inoculated media are loaded on conveyors for rapid transfer between WASP and

automated incubators where cultures are read with high-resolution digital imaging at pre-defined times. Consequently, phenotypic AST should benefit from the automation, particularly as the inoculation of agar plates represents one of the most critical steps that could affect the accuracy and repeatability of the disc diffusion method. Workload requirements for the disc diffusion method typically preclude their routine use in many clinical microbiology laboratories. However, the WASPLab was capable of performing AST by disc diffusion with an accuracy equal to or greater accuracy than that of the SIRscan, and with lower marginal costs than other commercially available methods. We defined the timeliness as the time required to inoculate agar plates manually, to apply discs, and to transport and to load the inoculated plates in the SIRscan 2000 automatic by the technician. We estimated that the WASPLab permitted halve the technician time required for performing a high number of AST by disc diffusion. Further improvements, such as automated preparation of the bacterial inoculum, will probably offer even greater benefits.

Although our study was set up to evaluate the effectiveness of the WASPLab to perform AST by disc diffusion and not specifically to explain the differences between the WASPLab incorporating the BioRad expert system and the SIRscan 2000 automatic, we confirm that most of the major errors observed in this study may be due to inadequate quality of antibiotic-impregnated discs.

The present study has the following limitations: we did not include multidrug-resistant strains such as carbapenemase-producing *Enterobacteriaceae*, and we used antibiotic-impregnated discs from two different manufacturers.

## Conclusions

In summary, the WASPLab incorporating the BioRad expert system provides a fully automated solution for antimicrobial disc diffusion susceptibility testing with accuracy that is equal to or better than the accuracies of other available phenotypic methods. In this study, we used the same incubation times for both systems (16 h) so that the effects on the turn-around time remain marginal. In contrast, most AST results can now be reported 1 day earlier with the implementation of the EUCAST rapid AST directly from positive blood-culture bottles and from isolated clinical strains according to previously published protocols [13,14]. Additional automation (e.g. by automating the preparation of the bacterial inoculum) will further reduce workload requirements and time associated with the disc diffusion method.

## Transparency declaration

The authors declare that the research was conducted in the absence of any commercial or financial relationships in the last 3 years that could be construed as a potential conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2019.11.008>.

**Table 5**  
Major errors and very major errors observed with the two compared methods

	No. of strains	Antibiotics tested by strain	Method	Major error	Very major error
<i>Staphylococcus Aureus</i>	94	11	WASPLab	4	0
<i>Pseudomonas Aeruginosa</i>	67	12	SIRscan	1	0
<i>Enterobacteriales</i>	177	16	WASPLab	1	0
			SIRscan	3	3
			WASPLab	26	0
			SIRscan	3	5

## References

- [1] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966;45:493–6.
- [2] Mulvey MR, Simor AE. Antimicrobial resistance in hospitals: how concerned should we be? *CMAJ* 2009;180:408–15.
- [3] Ruppé E, Cherkaoui A, Charretier Y, Girard M, Schicklin S, Lazarevic V, et al. From genotype to antibiotic susceptibility phenotype in the order *Enterobacterales*: a clinical perspective. *Clin Microbiol Infect* 2019 Oct 3. <https://doi.org/10.1016/j.cmi.2019.09.018>. [Epub ahead of print]. PMID: 31586657.
- [4] Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, et al. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST subcommittee. *Clin Microbiol Infect* 2017;23:2–22.
- [5] Stoesser N, Batty EM, Eyre DW, Morgan M, Wyllie DH, Del Ojo Elias C, et al. Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae* isolates using whole genomic sequence data. *J Antimicrob Chemother* 2013;68:2234–44.
- [6] Gordon NC, Price JR, Cole K, Everitt R, Morgan M, Finney J, et al. Prediction of *Staphylococcus aureus* antimicrobial resistance by whole-genome sequencing. *J Clin Microbiol* 2014;52:1182–91.
- [7] Bailey AL, Ledebner N, Burnham CD. Clinical microbiology is growing up: the total laboratory automation revolution. *Clin Chem* 2019;65:634–43.
- [8] Cherkaoui A, Renzi G, Charretier Y, Blanc DS, Vuilleumier N, Schrenzel J. Automated incubation and digital image analysis of chromogenic media using Copan WASPLab enables rapid detection of vancomycin-resistant *Enterococcus*. *Front Cell Infect Microbiol* 2019 Nov 6;9:379. <https://doi.org/10.3389/fcimb.2019.00379>. eCollection 2019.
- [9] Cherkaoui A, Renzi G, Vuilleumier N, Schrenzel J. Copan WASPLab automation significantly reduces incubation times and allows earlier culture readings. *Clin Microbiol Infect* 2019 Nov;25(11):1430.e5–1430.e12. <https://doi.org/10.1016/j.cmi.2019.04.001>. Epub 2019 Apr 12. PMID: 30986560.
- [10] Hombach M, Maurer FP, Pfiffner T, Bottger EC, Furrer R. Standardization of operator-dependent variables affecting precision and accuracy of the disk diffusion method for antibiotic susceptibility testing. *J Clin Microbiol* 2015;53:3864–9.
- [11] Hombach M, Ochoa C, Maurer FP, Pfiffner T, Bottger EC, Furrer R. Relative contribution of biological variation and technical variables to zone diameter variations of disc diffusion susceptibility testing. *J Antimicrob Chemother* 2016;71:141–51.
- [12] Baker CN, Thornsberry C, Hawkinson RW. Inoculum standardization in antimicrobial susceptibility testing: evaluation of overnight agar cultures and the rapid inoculum standardization system. *J Clin Microbiol* 1983;17:450–7.
- [13] Hombach M, Jetter M, Blochliger N, Kolesnik-Goldmann N, Bottger EC. Fully automated disc diffusion for rapid antibiotic susceptibility test results: a proof-of-principle study. *J Antimicrob Chemother* 2017;72:1659–68.
- [14] Hombach M, Jetter M, Blochliger N, Kolesnik-Goldmann N, Keller PM, Bottger EC. Rapid disc diffusion antibiotic susceptibility testing for *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterococcus* spp. *J Antimicrob Chemother* 2018;73:385–91.