WASP™ and WASPLab™ Fully Automated Disk Diffusion Antimicrobial Susceptibility Testing (AST): from Streaking to Interpretation

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INTRODUCTION:

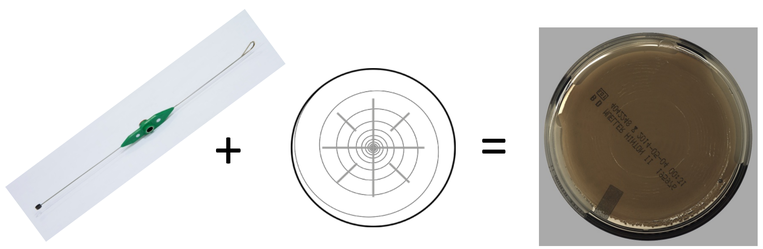
Disk diffusion is the original methodology for antimicrobial susceptibility testing and remains one of the most widely used in routine clinical microbiology laboratories. However, the results can be highly influenced by manual plate preparation and subjectivity of inhibition zone reading. WASP™, an automated system able to process clinical samples, is connected to WASPLab™, a system that incorporates an automated incubator and an image module able to capture and digitalize plate images, allowing automated software analysis.

OBJECTIVES:

The objectives of this study were to validate:
- WASP-WASPLab™ fully automated AST procedure:
- WASP™ plates preparation in compliance with EUCAST recommendations
- WASPLab™ automated measurement of inhibition zone diameters and analysis by an integrated expert system that finalizes the report.

MATERIALS & METHODS :

In this study the optimal inoculum volume, bacterial concentration and streaking conditions were determined in order to obtain results comparable to manually prepared plates. A WASP™ AST dedicated spreader was developed to obtain a homogeneous distribution of bacterial suspension on Müller-Hinton agar plates.



Antibiotic disks were automatically deposited by WASP™ and automatically incubated in WASPLab™.

Digitalized plates were then analysed by WASPLab™ software and antibiotic inhibition zone were defined. In order to validate the performance of automated AST, 4 EUCAST recommended QC aerobic strains (*E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 and *E. faecalis* ATCC 29212), and 50 aerobic clinical isolates were used with a wide combination of antibiotics. The inhibition zones of WASP™ prepared plates were automatically measured by WASPLab™ and compared to inhibition zones of manually prepared plates measured with a calliper. Inhibition zones were analysed by the integrated expert system.

RESULTS:

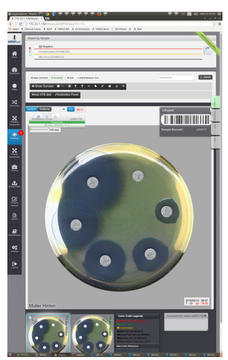
WASP™ plate preparations were in compliance with EUCAST recommendation for AST disk diffusion: even bacteria growth on the plate and 15+15+15 time slot for streaking, deposition and incubation. All readings of the 4 ATCC strains with both automated and manual reading systems were in compliance with EUCAST QC ranges. WASP™-WASPLab™ results were more precise and accurate: the standard deviations of automatically processed plates were on the overall lower than manually processed plates and closer to the EUCAST target.

	E. coli																							
	AK 30 µg Amikacin		ATM 30 µg Aztreonam		C 30 µg Chloraphenicol		MEM 10 µg Meropenem		NA 30 µg Nalidixic acid		F 100 µg Nitrofurantoin		CIP 5 µg Ciprofloxacin		TGC 15 µg Tigecycline		SXT 25 µg Trimethoprim-sulfamethoxazole		FOX 30 µg Cefoxitin		AMC 30 µg Amoxicillin clavulanic acid		FEP 30 µg Cefepime	
	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S
AV	23,6	22,74	31,6	32,55	24,88	25,08	30,88	30,79	24,92	25,02	20,82	19,44	33,73	35,94	22,21	22,47	23,33	24,82	24,23	25,48	20,71	20,35	32,12	31,33
SD	0,2	0,09	0,39	0,36	0,77	0,6	1,18	0,58	0,44	0,14	0,88	0,5	0,35	0,16	0,27	0,07	0,42	0,18	0,59	0,18	1,11	0,09	0,47	0,21
	22-23 (19-26)		32 (28-36)		24 (21-27)		31 (28-34)		25 (22-28)		20 (17-23)		35 (30-40)		23-24 (20-27)		26 (23-29)		26 (23-29)		21 (18-24)		34 (31-37)	

	S. aureus																							
	CN 10 µg Gentamicin		AMP 2 µg Ampicillin		FOX 30 µg Cefoxitin		LNZ 10 µg Linezolid		MI 30 µg Minocycline		TGC15 µg Tigecycline		DA 2 µg Clindamycin		E 15 µg Erythromycin		SYN 15 µg Quinupristin		NOR 10 µg Norfloxacin		SXT 25 µg Trimethoprim-sulfamethoxazole		LEV 5 µg Levofloxacin	
	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S
AV	21,94	21,63	18,4	18,47	26,22	27,17	23,41	24,76	25,42	26,03	21,17	22,43	25,66	26,4	25,93	25,42	25,13	24,69	21,43	23,22	29,52	29,6	24,7	25,86
SD	0,51	0,22	0,3	0,27	0,72	0,37	0,27	0,09	0,35	0,73	0,61	0,69	0,56	0,25	0,3	0,27	0,33	0,28	0,76	0,14	0,37	0,14	0,79	0,67
	22 (19-25)		18 (15-21)		27 (24-30)		24 (21-27)		26 (23-29)		22 (19-25)		26 (23-29)		26 (23-29)		24 (21-27)		21 (18-24)		29 (26-32)		26 (23-29)	

	E. faecalis																			
	AMP 2 µg Ampicillin		LNZ 10 µg Linezolid		CN 30 µg Gentamicin		IPM 10 µg Imipenem		F 100 µg Nitrofurantoin		TEC 30 µg Teicoplanin		TGC15 µg Tigecycline		SYN 15 µg Quinupristin		SXT 25 µg Trimethoprim-sulfamethoxazole		CIP 5 µg Ciprofloxacin	
	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S
AV	18,39	17,15	21,38	20,68	14,2	14,99	25,9	27,48	20	21,02	17,7	18,32	25,32	25,08	11,77	11,66	26,8	27,42	20,97	21,48
SD	0,51	0,15	0,41	0,22	0,35	0,3	0,51	0,4	0,5	0,46	0,37	0,27	0,27	0,33	0,45	0,26	0,26	0,033	0,76	0,26
	18 (15-21)		22 (19-25)		15 (12-18)		27 (24-30)		21 (18-24)		18 (15-21)		23 (20-26)		14 (11-17)		30 (26-34)		22 (19-25)	

	P. aeruginosa															
	AK 30 µg Amikacin		FEP 30 µg Cefepime		IPM 10 µg Imipenem		TPZ36 µg Piperacillin tazobactam		ATM 30 µg Aztreonam		LEV 5 µg Levofloxacin		CT 10 µg Colistin		TOB 10 µg Tobramycin	
	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S
AV	23,73	23,19	28,41	27,25	23,95	23,95	27,98	26,65	28,41	27,68	24,4	23,37	14,53	14,75	22,43	22,64
SD	0,64	0,11	0,7	0,28	0,66	0,64	0,91	0,03	0,49	0,13	1,18	0,2	0,43	0,42	0,63	0,21
	22 (18-26)		27 (24-30)		24 (17-23)		26 (23-29)		26 (23-29)		22-23 (19-26)		clsi 11-17		23 (20-26)	



O Reading by Operator
S Software Reading

For the 50 clinical isolates, the results of inhibition zone measurements according to clinical categorization displayed a 98% concordance between the automated and the manual processed. The 2% discrepancy (less then 2mm) was associated to specific combination of antibiotic and strains as Levofloxacin with *P. aeruginosa* causing a shifting from Intermediate to resistant.

Species	MI 30µg Minocycline		TE 30 µg Tetracycline		FOX 30 µg Cefoxitin		CIP 5 µg Ciprofloxacin		LEV 5µg Levofloxacin		CN 10µg Gentamicin		SXT 25µg Trimet-suf		FEP 30µg Cefepime		CAZ 10µg Ceftazidime		MEM 10 µg Meropenem		TPZ 36µg Pip-taz		CPD 30µg Cefpodoxime		CRO 30 µg Ceftriaxone		AMP 10µg Ampicillin	
	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S
E. coli	16	16	20	19	25	22	16	15	13	14	23	21	6	6	31	28	28	27	34	29	23	24	23	21	33	28	15	19
E. coli (ESBL)	14	16	6	6	21	22	6	6	6	6	20	20	6	6	11	9	6	9	29	28	6	6	6	6	6	6	6	6
E. coli	22	23	23	23	25	25	38	34	36	32	21	19	29	27	32	29	27	22	31	31	26	27	26	24	31	28	15	20
E. coli	19	20	23	21	24	22	37	32	34	30	23	20	6	6	28	27	24	22	32	29	18	18	23	21	29	27	6	6
E. coli	15	17	6	6	25	23	6	6	6	6	20	18	6	6	31	28	26	23	35	32	16	18	22	22	30	26	6	6
E. coli	20	19	22	24	22	24	37	33	37	29	23	19	25	27	32	31	26	26	30	29	24	26	22	26	30	29	21	13
E. coli	22	21	23	22	24	24	37	32	35	29	22	20	27	25	30	28	24	22	33	30	24	25	24	22	32	27	18	18
E. coli	10	10	6	6	25	24	37	33	34	31	22	21	6	6	32	30	27	24	32	30	25	25	25	23	32	28	6	6
E. coli (ESBL)	21	21	23	22	25	24	29	27	29	25	6	6	24	26	24	21	24	22	35	29	26	25	6	6	11	6	6	6
E. coli	20	21	21	22	25	24	37	37	35	33	21	20	26	26	31	28	25	22	33	30	24	24	25	22	31	27	19	18
E. coli	20	21	22	22	23	24	38	34	36	32	21	20	26	26	33	30	26	24	34	31	26	26	26	23	32	28	18	19
K. pneumoniae	18	18	23	22	21	23	32	30	30	28	20	19	22	22	31	30	27	25	28	27	23	23	27	26	30	28	6	7
K. pneumoniae	18	18	22	21	24	23	31	27	29	27	21	19	6	6	29	30	27	24	30	27	23	24	27	26	29	28	6	9
K. pneumoniae	17	18	20	21	24	23	31	29	30	28	22	19	24	24	31	30	26	24	32	29	22	22	26	25	30	28	6	6
K. pneumoniae	18	19	22	19	24	24	33	31	31	29	22	20	22	21	29	29	27	26	28	27	23	24	26	26	30	29	6	6

	CIP 5 µg Ciprofloxacin		LEV 5µg Levofloxacin		TOB 10µg Tobramycin		CN 10µg Gentamicin		FEP 30µg Cefepime		IPM 10µg Imipenem		MEM 10 µg Meropenem		TPZ 36µg Pip-taz						LEV 5µg Levofloxacin		CN 30µg Gentamicin		AMP 2µg Ampicillin		VA 5µg Vancomycin	
	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S					O	S	O	S	O	S	O	S
P. aeruginosa	29	28	17	16	22	23	21	21	23	25	26	28	20	23	18	21			*E.faecalis*		21	22	6	6	18	17	17	15
P. aeruginosa	30	27	18	18	22	24	20	20	22	23	27	22	21	17	16	17			*E.faecalis*		20	20	17	18	18	19	16	13
P. aeruginosa	37	38	32	32	29	26	28	27	29	31	30	29	35	35	24	30			*E.faecalis*		23	24	15	16	15	16	16	13
P. aeruginosa	23	25	16	17	24	22	20	21	26	27	26	24	28	26	23	26			*E.faecalis*		6	6	20	20	6	6	19	18
P. aeruginosa	36	36	32	30	23	21	23	19	28	27	26	27	32	32	27	27			*E.faecalis*		6	6	6	6	6	6	19	18
P. aeruginosa	33	36	28	31	21	23	20	22	27	28	27	25	33	29	27	28			*E.faecalis*		6	6	6	6	6	7	19	18
P. aeruginosa	32	34	27	26	20	18	17	16	28	26	26	25	29	26	24	26			*E.faecalis*		6	6	19	20	6	8	18	17
P. aeruginosa	32	36	27	28	21	19	18	18	27	26	27	25	32	29	24	26			*E.faecalis*		17	20	21	21	6	6	19	18
P. aeruginosa	35	36	30	30	23	22	20	19	25	25	28	27	36	33	23	26												

	MI 30µg Minocycline		TE 30 µg Tetracycline		E 15µg Erythromycin		DA 2µg Clindamycin		FOX 30 µg Cefoxitin		P 1u Penicillin		CIP 5 µg Ciprofloxacin		LEV 5µg Levofloxacin		RA 5µg Rifampicin		CN 10µg Gentamicin		SXT 25µg Trimet-suf							
	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S	O	S						
S. aureus	29	34	27	34	6	6	6	6	27	26	12	12	6	6	9	10	34	34	25	26	30	30						
S. aureus	27	30	27	32	26	28	27	28	27	26	13	15	28	28	31	31	36	33	22	22	29	30						
S. aureus	28	29	29	30	27	27	28	28	26	27	12	13	29	27	30	30	32	31	23	23	27	30						
S. aureus	27	30	28	31	29	27	29	28	26	28	11	11	30	29	32	32	36	33	23	23	28	30						
S. aureus	29	29	28	29	27	27	27	28	24	28	14	13	31	32	32	33	38	34	23	22	29	28						
S. aureus	26	29	27	31	27	27	26	27	26	28	11	10	27	26	28	28	34	32	22	22	28	28						
S. aureus	27	29	28	30	26	27	25	27	27	30	34	34	24	24	28	27	36	31	21	22	29	31						
S. aureus	29	29	29	30	27	27	29	27	25	24	11	10	30	28	31	31	35	32	23	23	29	30						
S. aureus	27	29	27	30	29	27	28	28	27	28	34	34	30	26	30	32	34	31	22	21	27	29						
S. aureus	28	29	28	30	28	26	27	26	28	31	35	34	29	26	31	32	34	30	22	21	27	29						
S. aureus	27	29	26	30	26	27	27	28	27	30	36	36	29	27	30	31	35	31	22	24	26	30						
CoNS	31	30	31	27	33	28	33	30	15	13	9	8	13	11	15	15	38	35	26	25	29	27						
CoNS	28	26	8	9	15	16	31	27	30	29	21	14	31	30	31	31	40	39	31	27	28	26						
CoNS	26	27	27	27	6	6	6	6	6	6	6	6	14	14	19	19	6	6	6	6	6	6						
CoNS	27	29	29	31	6	6	6	10	6	16	6	8	6	8	11	15	6	6	6	6	6	6						

Resistant | Susceptible | Intermediate

O Reading by Operator
S Software Reading

CONCLUSION:

Fully automated WASP™-WASPLab™ AST provides results in compliance with EUCAST guidelines, reduces manual workload, and eliminates readings subjectivity resulting in a standardized AST performance

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