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ORIGINAL ARTICLE

Performance of rapid antimicrobial susceptibility testing by disk diffusion on MHR-SIR agar directly on urine specimens

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Abstract

The standard method for the diagnosis of urinary tract infections is urine culture that requires 18–48 h for the identification of the bacteria and an additional 24 h until the results of antimicrobial susceptibility testing (AST) are available. We evaluated here a rapid AST method by disc diffusion performed directly on urine samples with a delay of 8 h. A total of 245 urine samples with monobacterial Gram negative observed on microscopy were tested in parallel by two AST methods. Rapid AST method was performed directly on urine samples using Rapid Mueller-Hinton (MHR-SIR) with 8-h incubation before reading and standard method was performed as usual. We compared the categorical agreement and the correlation between the diameters obtained by standard method and by MHR-SIR directly on urine samples. Over the 5285 tested combinations, we observed 5172 (97.9%) categorical agreement, 82 (1.5%) minor errors, 17 (0.3%) major errors, and 14 (0.3%) very major errors. Our results showed an excellent categorical agreement and correlations between diameters for MHR-SIR and standard methods. MHR-SIR performed directly on urine samples with monomicrobial *Enterobacteriacae* can predict the result of overall AST profile in 8 h with reliable results. The main advantage of MHR-SIR is that it offers the possibility of obtaining results 40 h earlier than conventional AST. The cost is estimated for less than 6 USD for 16 antibiotics, chosen by the microbiologist.

Keywords MHR-SIR · Direct AST · Rapid AST · Urinary tract infections

Highlights

- First evaluation of Mueller-Hinton Rapid-SIR agar (MHR-SIR) directly from urine specimens
- Time gain of 40 h compared to standard methods with a read of a non-expert microbiologist
- This study showed excellent categorical agreement and correlations between diameters for MHR-SIR and standard MH methods.
- In the context of increasing antimicrobial resistance among Gram-negative bacteria, MHR-SIR allow early antibiotic appropriateness in 8 h.
- MHR-SIR on urine can make an important contribution to patient management and reduce the use of broad-spectrum antimicrobial agents.

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Introduction

For the diagnosis of urinary tract infections, classical laboratory methods require a delay of 48 h minimum according to recommendations [1]. This is the time necessary to obtain the identification, culture enumeration, and the antimicrobial susceptibility testing (AST) of the bacteria [2]. In the context of bacterial resistances [3–5], decreasing the delay for obtaining AST results is a key to avoid therapeutic failures. In clinical microbiology laboratories, the disk diffusion method performed after culture described by Bauer et al. in 1966 [6] is widely used for AST, with 16 to 24 h of incubation recommended by EUCAST [7]. Since the 1970s, several studies tested direct antimicrobial susceptibility testing (DST), directly from urine samples [8–12]. Results showed good concordances between the standard method and the DST method, particularly if the bacterial inoculum is sufficient and if the culture is monomicrobial. Recent studies have demonstrated that the incubation time of Mueller-Hinton agar could be reduced [13–17] to accelerate AST. In our study, we demonstrated that DST can be determined directly on urine samples on MHR-SIR (Rapid Mueller-Hinton) coupled with an

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automatic reading using SIRscan® 2000 Automatic system (i2a, France) with concordant results, to obtain an AST with a delay of 8 h.

Material and methods

Specimen collection

This prospective pilot study was carried out in a routine clinical microbiology laboratory between August 2016 and April 2017. Cytobacteriological examination of the urine was performed by flow cytometry (Sysmex UF-500i, bioMérieux, France) and followed by Gram stain. Urine specimens were included if leukocyturia $> 5.10^4$/mL with monomicrobial presence of Gram-negative bacteria on microscopic examination.

Disk diffusion testing

Two AST methods were performed in parallel on samples meeting the inclusion criteria detailed above: the standard method and the rapid method. The standard method was performed on Mueller-Hinton agar (Bio-Rad Laboratories, France) incubated for 16 h from a colony obtained after an overnight subculture on CPS agar (bioMérieux, France). The rapid method was performed by direct inoculation using a swab on MHR-SIR agar (i2a, France), as recommended by the British Society for Antimicrobial Chemotherapy (BSAC) [18]. Inhibition zones were read from digital images with the SIRscan® 2000 Automatic system (i2a, France) after 8 h of incubation and were interpreted using CASFM-EUCAST 2015 breakpoints [1].

Discrepancies

For each bacterium and each antibiotic, we compared the concordance of interpretation between the two methods: susceptible (S), intermediate (I) or resistant (R). The discrepancies were classified as follows: minor error, major error (ME), and very major error (VME). Strains interpreted "S" or "I" with a method and respectively "I" or "R" with the other method were classified as minor errors. The major errors (ME) represented the strains interpreted "R" with MHR-SIR method and "S" with the standard method. The very major errors (VME) represented the strains interpreted "S" with MHR-SIR method and "R" with the standard method. The diameters obtained by the two methods were compared and correlation coefficients r were assessed with Pearson test for each antibiotic tested. In case of discrepancies (ME and VME) between results of inhibition zones with MH and MHR-SIR agar, minimum inhibitory concentration (MIC) was determined restrospectively from − 80 °C conserved frozen Extended spectrum β-lactamase (ESBL)-producing strains by ETEST® method (bioMérieux, La Balme-Les-Grottes, France).

Results

During this period, 321 isolates from urine samples with *Enterobacteriaceae* were preselected on the defined criteria and 245 samples were included. They were distributed as 193 (79%) *Escherichia coli*, 20 (8%) *Klebsiella pneumoniae*, 14 (6%) *Proteus mirabilis*, 9 (4%) *Enterobacter cloacae*, 4 (2%) *Citrobacter koseri*, 3 (1%) *Klebsiella oxytoca*, 1 (< 1%) *Raoultella planticola*, and 1 (< 1%) *Serratia marcescens*. Concerning the 76 excluded urine samples, 32 urines had a discordant culture from the direct exam (10%), 28 were polymicrobial with two or more GNR present in the culture (8.7%) and 16 were unreadable after 8 h of culture because of an insufficient growth (5%) (Fig. 1). Among the strains of *Enterobacteriaceae* studied, different mechanisms of beta-lactam resistance were observed by phenotypic analysis. The population comprised of 53% wild-type strains for beta-lactams (n = 130), 21% acquired penicillinase producers (n = 52), 10% ESBL producers (n = 23), 14% inhibitor-resistant TEM [IRT] (n = 35), and 2% other mechanisms (e.g., AmpC) (n = 5). No carbapenemase production was observed among the tested strains.

Over the 5285 tested combinations, there were 5172 (97.9%) categorical agreement, 82 (1.5%) minor errors, 17 (0.3%) ME, and 14 (0.3%) VME (Table 1).

Minor errors were mainly reported with quinolones (nalidixic acid, ofloxacin, and ciprofloxacin) with respectively 7 (2.9%), 14 (5.7%), and 11 (4.5%) minor errors (illustrated on Fig. 2). Focusing on comparison of diameters between the two methods, we described correlation coefficients $r > 0.90$ for ofloxacin, nalidixic acid, and ciprofloxacin (Table 1). Both piperacillin-tazobactam and trimethoprim-sulfamethoxazole counted 10 minor errors, which represented respectively 4.3 and 4.1% (Table 1).

Concerning the major errors, 8 ME were observed for amoxicillin-clavulanic acid (3.5%) and 6 ME for temocillin (2.7%).

Of the 17 very major errors observed, cephalexin was the cause of most VME (9 VME, 3.7%). Of the 9 VME for cephalexin, 8 involved AmpC cephalosporinase producing *Escherichia coli* (Fig. 2) and 1 *Enterobacter cloacae*.

Focusing on the 23 resistant strains producing ESBL, corresponding to 506 combinations of antibiotic-bacteria, we observed the following results: n = 468 (92.5%) categorical agreement, n = 33 (6.5%) minor errors, n = 3 (0.6%) ME, and n = 2 (0.4%) VME. Determination of MIC by ETEST® in case of ME and VME strains showed that MIC matched alternatively with MH and MHR-SIR results (Table 2).

Discussion

Our results show an excellent correlation between diameters obtained with standard MH and MHR-SIR. According to the

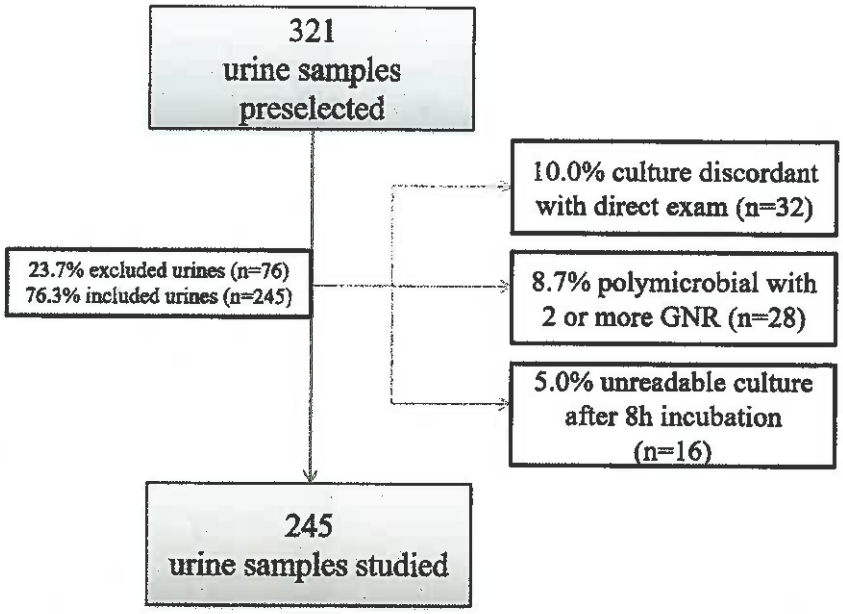

Fig. 1 Urine samples studied. Over the 321 urines preselected, 23.7% were excluded by the selection criteria: discordance between direct exam and culture; several GNR observed in the culture; culture unreadable after 8 h of incubation despite an adequate culture with standard MH method

FDA and Jorgensen [19, 20], the concordance is acceptable. Indeed, the results show 97.9% of categorical agreement, 1.5% of minor errors, 0.3% ME, and 0.3% VME. According to our results, cephalexin (3.7% of VME) does not correlate

Table 1 Concordances of MHR-SIR results (8 h) compared to standard MH method (16 h) on *Enterobacteriaceae* in urine samples

	Total	CA		Minor errors		ME		VME		r
		n	%	n	%	n	%	n	%	
Amoxicillin	243	243	100.0	0	0.0	0	0.0	0	0.0	0.96
Ticarcillin	242	242	100.0	0	0.0	0	0.0	0	0.0	0.97
Cephalexin	245	236	96.3	0	0.0	0	0.0	9	3.7	0.87
Ertapenem	244	242	99.2	2	0.8	0	0.0	0	0.0	0.39
Cefotaxime	242	239	98.8	3	1.2	0	0.0	0	00	0.88
Amoxicillin - clavulanic acid	234	224	95.7	0	0.0	8	3.5	2	0.8	0.89
Ceftazidime	245	239	97.6	6	2.4	0	0.0	0	0.0	0.83
Imipenem	241	240	99.6	1	0.4	0	0.0	0	0.0	0.22
Piperacillin - tazobactam	230	220	95.7	10	4.3	0	0.0	0	0.0	0.69
Cefepime	244	236	96.7	8	3.3	0	0.0	0	0.0	0.73
Trimethoprim - sulfamethoxazole	242	232	95.9	10	4.1	0	0.0	0	0.0	0.94
Gentamicin	243	241	99.2	2	0.8	0	0.0	0	0.0	0.89
Amikacin	239	238	99.6	1	0.4	0	0.0	0	0.0	0.22
Nalidixic acid	245	238	97.1	7	2.9	0	0.0	0	0.0	0.93
Ofloxacin	244	228	93.4	14	5.7	2	0.9	0	0.0	0.90
Ciprofloxacin	243	231	95.1	11	4.5	1	0.4	0	0.0	0.91
Cefixime	237	236	99.6	0	0.0	0	0.0	1	0.4	0.88
Nitrofurantoin	238	237	99.6	0	0.0	0	0.0	1	0.4	0.75
Mecillinam	239	239	100.0	0	0.0	0	0.0	0	0.0	0.74
Temocillin	230	223	96.9	0	0.0	6	2.7	1	0.4	0.64
Cefoxitin	235	230	97.9	5	2.1	0	0.0	0	0.0	0.75
Fosfomycin	240	238	99.2	2	0.8	0	0.0	0	0.0	0.65
Total	5285	5172	97.9	82	1.5	17	0.3	14	0.3	

CA categorical agreement, ME major error, VME very major error, r correlation coefficient

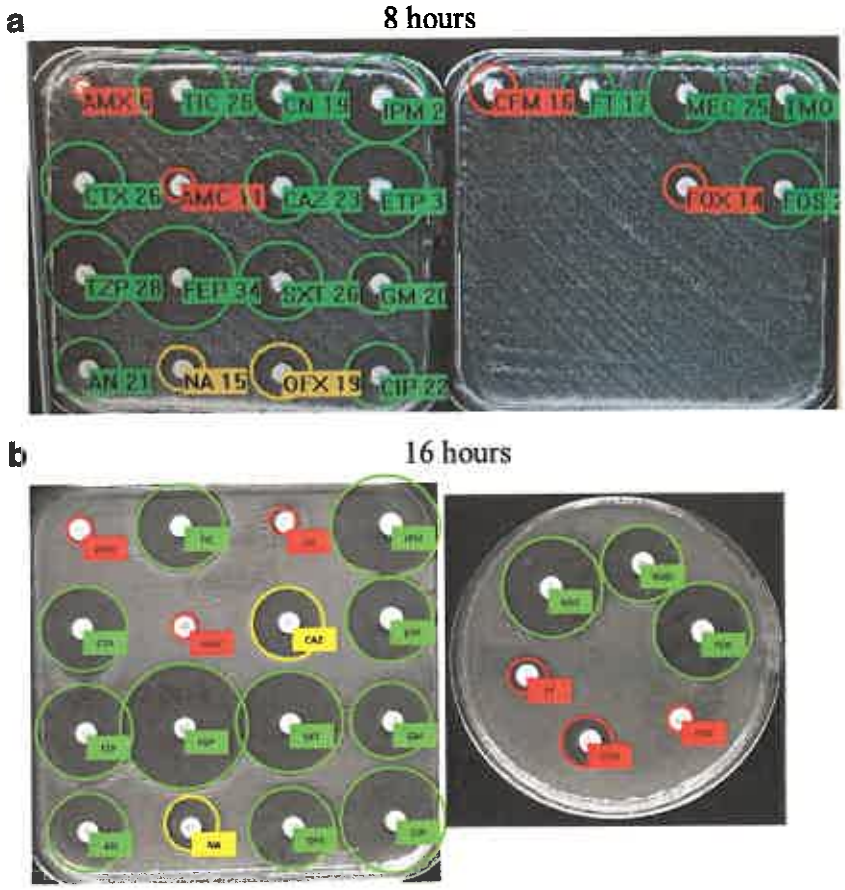

Fig. 2 SIRscan® 2000 Automatic photos of an AmpC hyperproducer *Escherichia coli* strain isolated from urine sample on MHR-SIR (a) and standard MH (b) after respectively 8-and 16-h incubation. The photograph illustrates the AST profile of an *E. coli* strain. Two very major errors are visible around cephalexin and nitrofurantoin. Resistance to cephalexin is detected on standard MH after 16 h of incubation (b.) but is not detected after 8 h of culture on MHR-SIR media (a). Two minor errors are visible around ceftazidime and ofloxacin. AMX amoxicillin, TIC ticarcillin, CN cephalexin, ETP ertapenem, CTX cefotaxime, AMC amoxicillin – clavulanic acid, CAZ ceftazidime, IPM imipenem, TZP piperacillin – tazobactam, FEP cefepime, SXT trimethoprim – sulfamethoxazole, GM gentamicin, AN amikacin, NA nalidixic acid, OFX ofloxacin, CIP ciprofloxacin, CFM cefixime, FT nitrofurantoin, MEC mecillinam, TMO temocillin, FOX cefoxitin, FOS fosfomycin

on MHR-SIR and does not allow cephalosporinase detection in 8 h, although this has no impact in clinical practice. Considering other antibiotics with high rates of errors, amoxicillin-clavulanic acid shows 3.6% VME and temocillin shows 2.7% ME, in the absence of a defined intermediate zone. These high rates of errors call for further discussion [20] and caution must be observed before drawing conclusions. A variation of one millimeter around the breakpoint diameter can lead to a ME or VME. Concerning amikacin, the correlation coefficient is weak ($r = 0.22$), even though neither major nor very major errors were detected; only one minor error was recorded. All the *Enterobacteriaceae* studied were susceptible to amikacin.

Nevertheless, our study has certain limitations. For bacterial growth, bacteria like *Pseudomonas aeruginosa* do not grow fast enough to allow reading of diameters after 8 h of incubation. That is a drawback of this solid method. In addition, we noticed a high rate of excluded urines, almost a quarter of all the samples. For 5% of urine samples, bacterial inoculum was too poor to allow a sufficient growth after 8 h of

Table 2 Determination of MIC for producing ESBL-producing strains showing major and very major differences, MIC were interpreted according to CASFM-EUCAST 2015 guidelines

Bacteria	Antibiotic	MHR-SIR result	MH result	Difference	MIC measure Etest (mg/L)	MIC cutoff* (mg/L) S ≤ R>	Interpretation MIC result	Correct method
E. coli	Amoxicillin-clavulanate	S	R	VME	4	8	S	MHR-SIR
E. coli	Amoxicillin- clavulanate	R	S	ME	12	8	S	MHR-SIR
K. pneumoniae	Ofloxacin	R	S	ME	0.5	0.5–1	S	MH
E. coli	Cefixime	S	R	VME	6	1	R	MH
K. oxytoca	Temocillin	S	R	VME	16	8	R	MH

S susceptible, I intermediate, R resistant, me minor error, ME major error, VME very major error, MH Mueller-Hinton, MHR Mueller-Hinton Rapid-SIR, MIC minimum inhibitory concentration

*CASFM-EUCAST 2015

culture with MHR-SIR. For 9% of urine samples, several *Enterobacteriaceae* were observed at the culture. These urines were excluded but results could still be clinically informative due to the overall information obtained in case of resistance. Despite some discrepancies, there are several benefits on the MHR-SIR method. AST by MHR-SIR is a rapid and reliable method for *Enterobacteriaceae* directly from urine samples. The overall sensitivity profile is obtained only 8 h after the urines were collected and the estimated cost is less than 6 USD $ for 16 antibiotics, chosen by the microbiologist. The two methods compared in our study were performed at equivalent costs because the disposable used were identical. We could envisage performing this technique directly on selected urine samples, with relevant clinical criteria. However, this must be performed in partnership with antimicrobial stewardship in order to adapt or initiate antibiotherapy the same day of the urine collection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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