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Evaluation of the Uro-Quick system for antibiotic susceptibility tests of strains collected from intensive care units

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Abstract - During the period January-June 2004, 525 pathogens isolated from intensive care units were examined with the new rapid Uro-Quick method for antibiotic susceptibility tests. The results were compared with those obtained by the reference NCCLS methods (disk diffusion or dilution). Antibiotic (in appropriate concentration) was introduced in a vial containing 2 ml of Mueller-Hinton broth, then 0.5 ml of 5 x 10^5 or 10^6 cells/ml of the strain culture were added. After 3-6 h of incubation, depending on the microorganism studied, the instrument printed the results: no growth and a growth curve similar to that of the untreated control are representative of a susceptible and resistant strain respectively. The following drugs were tested: ciprofloxacin, ampicillin, aztreonam, co-clavulanate, piperacillin/tazobactam, ceftazidime, cefotaxime, cefuroxime, ceftriaxone, imipenem, amikacin, gentamicin, trimethoprim-sulfamethoxazole, clindamycin, erythromycin, linezolid, penicillin, tetracycline, vancomycin, oxacillin. Gram-negative strains tested were 252 and Gram-positive 273: agreement between the two methods ranged from 85.6% (piperacillin/tazobactam) to 98.5% (ciprofloxacin) in Gram-negative pathogens, from 90 to 100% in Gram-positive, with the exception of erythromycin (84.2%) against enterococci. On the basis of the present findings the Uro-Quick system appears to be very useful for the rapid detection of antibiotic susceptibility in pathogens collected from intensive care units.

Key words: Uro-Quick, antibiotic susceptibility tests, disk-diffusion method, intensive care units.

INTRODUCTION

The increase resistance to antibiotics is a phenomenon widely distributed among a great variety of microorganisms (Howard *et al*., 2003). The driving force in the development of resistance has been recognized in the selective pressure exhibit by the drugs. Several solutions have been advocated to fight bacterial resistance and the development of new drugs and adequate surveillance programs appear the measures that capture a general consensus (Gastemeir, 2004; Magee *et al*., 2005). Surveys addressed to monitor the incidence of antimicrobial resistance in certain species as well as in different geographic areas are also need in order to provide microbiological data for the physicians because infections are seldom diagnosed on an etiologic basis (Gastmeier, 2004) even in hospitals. Therefore, the success of the empiric therapy adopted depends, not only, on the overall conditions of the patient, but also, on the ability of the physician to guess the pathogen and its resistance pattern. With the aim to support clinicians in a timely manner many automated methods have been developed and evaluated (Felmingham and Brown, 2001, Livermore *et al.*, 2002; Ferraro and Jorgen, 2003). A simple and a rapid test, if effective, could, in fact, increase laboratory efficiency, decrease costs, and allow physicians to start prompt therapy.

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The Uro-Quick, one of these automatic instruments, is widely used for the screening and determination of bacteriuria providing information for physicians in 3-5 hours (Breda, 1996; Iverson *et al.,* 1999; Branca *et al*., 2001). Recently the Uro-Quick system was employed to perform antibiotic susceptibility tests directly on urine samples (Debbia *et al*., 2004) and these preliminary results indicate an agreement higher than 90% with standard disk susceptibility tests. Therefore, in 6-8 hours a urine specimen can be processed for the quantitative growth and antibiotic susceptibility tests of the pathogen.

In this study the Uro-Quick system has been employed to assess the antibiotic susceptibility of 525 pathogens isolated from intensive care units (ICU) during the period January-June 2004. The results were compared with those obtained by the standard Kirby-Bauer technique or, when necessary, by the dilution method (NCCLS 2003a, 2003b).

A preliminary communication of this study has been presented at the 44th Meeting, Interscience Conference of Antimicrobial Agents and Chemotherapy, ASM, Washington, USA, 2004 (Roveta *et al*., 2004a).

MATERIALS AND METHODS

Bacterial strains. During a six months period (January-June 2004), 525 strains collected from intensive care units were studied for antibiotic susceptibility tests employing the standard disk diffusion method and compared with the Uro-

Quick system. Globally 252 Gram-negative and 273 Gram-positive pathogens were isolated. The first group of bacteria included 92 *Escherichia coli*, 36 *Klebsiella* spp., 40 *Pseudomonas aeruginosa*, 44 *Enterobacter* spp., 19 *Proteus mirabilis* and 21 others. Gram-positive strains included 202 *Staphylococcus* spp., 38 *Enterococcus* 20 *Streptococcus* spp. and 13 *Streptococcus pneumoniae*.

Escherichia coli ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 (mecA negative) and *S. aureus* ATCC 43300 (mecA positive), *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299, were included as quality control strains.

Antibiotics. Towards the Gram-negative bacteria ciprofloxacin, nitrofurantoin, amoxicillin-clavulanate, ceftazidime, fosfomycin, imipenem, amikacin, trimethoprim-sulfamethoxazole, and piperacillin-tazobactam were tested. With respect to Gram-positive ciprofloxacin, nitrofurantoin, amoxicillin-clavulanate, ampicillin, fosfomycin, gentamicin, oxacillin, and trimethoprim-sulfamethoxazole were assayed. The antibiotic powders and the disks used were obtained from commercial sources (Sigma-Aldrich and bioMérieux, Milan, Italy, respectively). Sterile stock solutions were prepared following the instructions of the manufacturers. Every single drug was introduced to an Uro-Quick vial containing 2 ml of Mueller Hinton (MH) broth in order to reach the appropriate concentration. Table 1 shows the antibiotics employed in this study, their respective breakpoints according to National Committee for Clinical Laboratory Standards (NCCLS), (2003a) and the concentrations used in the Uro-Quick test-tube.

TABLE 1 – Antibiotics employed in this study, breakpoints and concentrations used in the Uro-Quick vial

Antibiotic[1]	Breakpoints[2] (µg/ml)	Concentrations[3] U.Q. (µg/ml)
Amikacin	16 - 32	20
Amoxicillin-clavulanate	8 - 32	15
Ampicillin (*Enterobacteriacee*)	8 - 32	10
Ampicillin (*Streptococcus pneumoniae*)	8 - 16	10
Aztreonoam	8 - 32	10
Cefoperazone	16 - 64	40
Cefotaxime (*Enterobacteriaceae*)	8 - 64	20
Cefotaxime (*S. pneumoniae*)	0.5	0.12-1
Cefotaxime(*Streptococcus viridans*)	0.5 - 2	1.25
Ceftazidime	8 - 32	20
Ceftriaxone	8 - 64	36
Cefuroxime	8 - 32	20
Ciprofloxacin (*Enterococcus* spp.)	1- 4	2
Ciprofloxacin	1-4	1
Clindamycin (*Streptococcus* spp.)	0.25 - 1	0.5
Clindamycin (*Staphylococcus* spp.)	0.5 - 4	2
Erythromycin (*S. pneumoniae*)	0.25 - 1	0.5
Erythromycin (*Staphylococcus* spp.)	0.5 - 8	1
Erythromycin (*Enterococcus* spp.)	0.5 - 8	0.5
Gentamycin	4 - 16	10
Imipenem (*Staphylococcus* spp.)	4 - 16	10
Imipenem (*Enterobacteriacea*e)	4 - 16	15
Linezolid (*Enterococcus* spp.)	2 - 8	5
Linezolid (S*taphylococcu*s spp.)	4	4
Linezolid (*Streptococcu*s spp.)	2	2
Oxacillin (S*taphylococcus aureus*)	2 - 4	3
Oxacillin (Coagulase negative staphylococci)	0.25 - 0.5	0.3
Penicillin	0.1	0.1
Penicillin (*S. pneumoniae*)	0.06 - 2	0.12 - 1
Piperacillin-tazobactam	16 - 128	72
Tetracycline (*Enterococcus* spp.)	4 - 16	10
Tetracycline (*S. pneumoniae*)	2 - 8	5
Trimethoprim-sulfamethoxazole	2 - 8	6
Trimethoprim-sulfamethoxazole (*S. pneumoniae*)	0.5 - 4	2
Vancomycin (*Enterococcus* spp.)	4 - 32	10
Vancomycin (S*taphylococcu*s spp.)	4	5

[1] When antibiotic concentrations were the same for all the species tested only a pair of breakpoint values was reported, instead, when the concentrations changed for the different species, more breakpoints values (with the single specie between brackets), were reported. [2] NCCLS (2003a). [3] The first number listed represents the susceptible breakpoint value and the second number indicates the resistant breakpoint value (as reported by NCCLS 2003a).

Susceptibility tests. The reference antimicrobial susceptibility tests used were the classic Kirby-Bauer disk diffusion method or, when necessary (i.e. to test fosfomycin), the dilution method using the procedures suggested by the NCCLS (2003a, 2003b). Strains were assigned to the susceptibility categories (susceptible, intermediate and resistant) after interpreting results according to the NCCLS (2003a) breakpoints.

The Uro-Quick system. The Uro-Quick system is an automated rapid method previously described (Breda, 1996). The samples are read every 5 minutes and the growth curves expressed in colony forming units (CFU/ml) are displayed on the screen prior to printing out after a specified reading time. Antimicrobial susceptibility tests were initially carried out as previously described (Debbia *et al.,* 2004) and after preliminary tests some parameters were modified. The appropriate concentration of antibiotic was introduced to a vial containing 2 ml of MH broth, a vial without the drug was used as control and the inoculum was prepared from a direct colony suspension. In detail, an aliquot (0.5 ml) of the bacterial samples was added to a vial containing the antibiotic solution to reach a final dose as those reported in Table 1 and then incubated in the instrument. The inoculum size was adjusted to about 1 x 10^6 CFU/ml with all the strains tested with the exception of the members of the *Enterobacteriaceae* family that were assessed using an inoculum of 5 x 10^5 CFU/ml. Streptococci were tested in cation adjusted Mueller-Hinton broth with 2% v/v lysed horse blood. *Pseudomonas aeruginosa* was routinely cultured in MH broth containing 2% KCl and 0.2% Tween 80. The vial caps were also removed to increase oxygenation of the medium. The growth rate of *P. aeruginosa* in this medium was found faster than in the usual MH without any other ingredients. Following the appropriate period of time, Uro-Quick detected bacterial growth (for resistant strains) or no growth (susceptible). The software was also modified by the manufacturer and the time of incubation of the samples was adapted for each antibiotic/bacterial species combination (from 3 to 24 h), however in a range from 3 to 6 h all the drug- resistant phenotypes were detected.

RESULTS

Results of susceptibility obtained on the quality control strains employing the NCCLS reference methods and using the Uro-Quick system were as expected.

In Table 2 are reported the percentages of concordance between the data obtained with the Uro-Quick system and the disk diffusion method on 252 Gram-negative microorganisms. In considering *Enterobacteriaceae*, agreement between the two methods was found in more than 90% of the cases with the exception of amoxicillin-clavulanate (89.0%), and pipracillin-tazobactam (85.8%). More in detail, *E. coli* showed concordance with the range from 90.2% (cefuroxime and aztreonam) to trimethoprim-sulfamethoxazole (100%) of the cases, while amoxicillin-clavulanate registered 89.1%.

TABLE 2 – Percentage of agreement between Uro-Quick and disk diffusion method in 252 Gram-negative pathogens.

Microorganisms (No. of strains)	Agreement (%)												
	AMP	AMC	TZP	CTX	CRO	CXM	CAZ	IPM	ATM	AN	GM	CIP	SXT
Enterobacteriaceae (191)	97.6	89.0	85.8	98.2	94.7	94.1	94.1	93.9	91.1	98.2	96.6	98.5	98.4
Escherichia coli (92)	92.4	89.1	93.8	100	97.8	90.2	95.6	97.5	90.2	95	97.8	96.7	100
Proteus mirabilis (19)	100	89.4	88.9	100	100	89.4	94.7	84.2	84.2	100	100	94.7	100
Klebsiella pneumoniae (26)	–(2)	88.4	100	100	100	100	100	100	100	100	100	100	100
Klebsiella oxytoca (10)	–	80	80	100	100	100	100	100	80	100	100	100	100
Enterobacter cloacae (26)	100	96.1	88.4	100	100	100	96.1	95.6	92.3	100	96.1	100	95.6
Enterobacter aerogenes (18)	100	94.4	88.8	93.7	88.8	88.8	100	100	88.8	93.7	100	100	100
Pseudomonas aeruginosa spp. (40)	–	–	90	–	–	–	92	92	93.4	97	100	97	–
Others(1) (21)	95.2	90.4	85.7	93.3	95.2	95.2	90.4	100	80.9	100	90.4	100	93.3

AMP: ampicillin; AMC: amoxicillin-clavulanate; TZP: piperacillin-tazobactam; CTX: cefotaxime; CRO: ceftriaxone; CXM: cefuroxime; CAZ: ceftazidime; IPM: imipenem; ATM: aztreonam; AN: amikacin; GM: gentamicin; CIP: ciprofloxacin; SXT: trimethoprim-sulfamethoxazole.
(1) *Citrobacter* spp., 6 strains (3 *C. freundii* and 3 *C. koseri*)*; Morganella morganii,* 3 strains; *Providencia stuartii,* 3 strains; *Serratia* spp.*,* 5 strains (3 *S. marcescens* and 2 *S. liquefaciens), Stenotrophomonas* spp.*,* 4 strains. (2) -: Not assayed.

TABLE 3 – Percentage of agreement between Uro-Quick and Kirby-Bauer method in 273 Gram positive pathogens

Microorganisms	Agreement (%)													
	OXA	PEN	AMP	CTX	CRO	IMP	ERI	DA	GM	LZD	TE	CIP	SXT	VA
Staphylococcus spp. (202)	98.8	95.5	–(1)		–	96.3	96.3	97.5	90.0	100	93.8	100	95.0	93.8
Enterococcus spp. (38)	–	–	100		–	–	84.2	–	–	100	92.1	94.5	–	94.5
Streptococcus spp. (20)	–	100	–	90	100	–	100	90	–	100	–	–	–	–
Staphylococcus pneumoniae (13)		92.3	–	100	100	–	100	92.3	–	100	100	–	100	–

OXA: oxacillin; PEN: penicillin; AMP: ampicillin; CTX: cefotaxime; CRO: ceftriaxone; IMP: imipenem; ERI: erythromycin; DA: clindamycin; GM: gentamicin; LZD: linezolid; TE: tetracycline; CIP: ciprofloxacin; SXT: trimethoprim-sulfamethoxazole; VA: vancomycin.
(1) -: Not assayed.

Proteus spp. showed a complete concordance with ampicillin, cefotaxime, ceftriaxone, amikacin, gentamycin, and trimethoprim-sulfamethoxazole. A similar favourable behaviour was noted with both ceftazidime and ciprofloxacin (94.7%), while the other antibiotics registered values of agreement from 84.2 to 89.4%.

Klebsiella spp. demonstrated 100% concordance with all the antibiotics, except with ampicillin (96.1%), amoxicillin-clavulanate (88.4%) and in few other cases with *K. oxytoca.*

Enterobacter spp. exhibited total concordance in tests with amikacin, cefotaxime, ceftazidime, ciprofloxacin and gentamycin. With other antibiotics percentages included between 85,7% and 92,9% were observed.

Pseudomonas aeruginosa registered with all the drugs evaluated agreement > 90% by the two methods the other strains demonstrated under all the experimental conditions an agreement >90.4% with the exception of piperacillin-tazobactam (85.7%) and aztreonam (80.9%).

In Table 3 there are results percentages between two systems about 273 Gram-positive strains.

When *Staphylococcus* spp. was studied with the two systems a concordance higher than 95.5% with all the antibiotics tested was registered with the exception of gentamicin (90.0%).

With *Enterococcus* spp. total concordance with ampicillin and linezolid occurred. High level of agreement was noted with vancomycin and ciprofloxacin (94.5%) as well as tetracycline (92.1%), while erythromycin registered 84.2% of concordance.

Streptococcus spp. demonstrated total agreement between two methods with all the antibiotics tested, in particular with penicillin, ceftriaxone, erythromycin and linezolid (100%). High level of accordance was also registered with cefotaxime and clindamycin (90%).

When *S. pneumoniae* was studied a total accordance by the two methods was exhibited by cefotaxime, cefuroxime, erythromycin, linezolid tetracycline and trimethoprim-sulfametoxazole. Penicillin and clindamycin fixed 92.3% of agreement.

CONCLUSIONS

The present findings demonstrate and confirm, on a large number of bacteria, that Uro-Quick system performed efficiently in the determination of the antibiotic susceptibility patterns in the Gram-negative and Gram-positive bacteria representing the most important pathogens collected from ICU, with an agreement >90% with the standard disk-diffusion method.

It is worth to underline that these strains are exposed to different classes of antibiotics that promote the development of a great variety of drug resistance phenotypes. A detailed analysis of the pathogens studied revealed that the discrepancies registered under all the experimental conditions adopted were mainly detected with borderline or intermediate susceptible strains. By mutation or exchange of genetic information microorganisms can rapidly evolve either to complete refractory or move toward resistance, against the major antibiotics, in a progressive manner from low to intermediate and finally to high levels (Tenover, 2001). New or unusual mechanisms of resistance might confer to bacteria a level of unsusceptibility to antibiotic that does not always increase the minimal inhibitory concentration of the strain high enough to exhibit resistance according to the breakpoint suggested by the NCCLS (2003a). Thus, when this microbial population is exposed to an antibiotic, the initial inoculum size, the drug concentration employed, and the period of time of incubation, might represent crucial points to move the balance toward no growth or proliferation of the culture. In this situation the reproducibility of the tests appear more difficult than in the other circumstances (Cockerill and Smith, 2004; Morens *et al*., 2004). Some factors that might influence the reproducibility of these tests were then analysed. The inoculum size was found more crucial for Gram-positive than for Gram-negative strains and it was, when necessary, adjusted to at least 10^6 CFU/ml with the first group of bacteria. The kinetic of growth was also identified as another parameter that influence the results, in particular, it was found that more reproducible results were obtained using exponentially growing bacteria for the inoculum. Finally, the effect of oxygenation was investigated, especially for tests involving aminoglycosides and fluoroquinolones, in these cases the cap of the vial was removed and, in the great majority of the cases, an increase in the concordance between the two systems was observed.

Furthermore, in a previous experience (Roveta *et al*., 2004b) it has been demonstrated that Uro-Quick correctly detected the antibiotic resistance phenotypes exhibited by a collection of well-characterised strains. Thus when the minimum inhibitory concentration of the resistant strains is distinctly different from that of susceptible microorganisms, the instrument easily detects the antibiotic susceptibility pattern of the pathogen. On the other hand with bacterial strains exhibiting phenotypes rarely or unusually encountered in diagnostic laboratories such as those that possess physiological perturbations due to the acquired resistance, Uro-Quick might give atypical results as any other automated or traditional techniques (Steward *et al*., 1999; Sanders *et al*., 2000; NCCLS, 2003a). Some of these aspects were considered during this phase of the study and the modifications adopted increased the incidence of agreement between the two methods.

These observations indicate that Uro-Quick system is flexible, easily and rapidly modifiable on the basis of pathogen and antibiotic considered suggesting a possible configuration of the software to any new situation. Under many circumstances this instrument has the potential to provide results in the same day that tests were set up.

Uro-Quick appears to be a reliable and promising instrument for the correct detection of antibiotic resistant pathogens.

The availability of the microbiological results in a timely manner, for the management of infections, can significantly reduce the empiric therapy in favour of a more direct treatment decreasing the use of inappropriate drugs and the diffusion of resistant pathogens especially in intensive care units where an appropriate and prompt therapy is required (Sanders *et al*., 2000; McGowan and Tenover, 2004).

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