# New broth - chromogenic Mueller Hinton agar procedure for urine samples – next-day result of *Enterobacteriaceae* antimicrobial susceptibility testing

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## Abstract

**Objectives:** The aim of this study was to compare antimicrobial susceptibility testing (AST) results of new rapid one-day procedure (RP) to results of standard two-day procedure (SP) for processing urine samples.
**Methods:** Routine urine samples were processed by SP and RP and results of AST for 15 antibiotics were compared.
SP: Urine was cultivated on chromogenic Uriselect 4 agar (Biorad, France) and isolated colonies were tested next day by standard CLSI disk-diffusion. Mueller Hinton agar (MHA - Biolife, Italy) and antibiotic disks (Becton Dickinson, USA) were used. Rapid methods or Vitek 2 (bioMerieux, France) were used for identification of isolates.
RP: Urine (0.5 mL) was inoculated into eugonic broth vials and incubated in HB&L Uroquattro (both Alifax, Italy) and incubated to turbidity at least Mc Farland 2.0 (3 to 6 hours). If only Gram-negative rods were seen on Gram stain, suspension was diluted to McFarland 0.5 (Densicheck, bioMerieux, France) and used as inoculum for CLSI disk-diffusion method, with two modifications: Chromatic MH agar (MHChr) (Liofilchem, Italy) was used and inhibition zones of mauve colonies on MHChr were measured against white background.
Only results of monomicrobial growth of *Enterobacteriaceae* were considered in this study. If growth of few enterococcal colonies on Uriselect 4 and MHChr occurred, it was neglected.
Differences between AST results of SP and RP were classified as minor errors (one result intermediate, other not), major errors (false resistance in RP) and very major errors (false susceptibility in RP).
**Results:** Two hundred isolates of *Enterobacteriaceae* were isolated (153 *Escherichia coli*, 19 *Klebsiella pneumoniae*, 15 *Proteus mirabilis*, 8 isolates of other enterobacterial species).
AST results of RP, compared to SP: from 3000 antibiotic results, 2900 results (96.7%) were in complete agreement. Rates of minor errors, major errors and very major errors were 2.8%, 0.4% and 0.2%, respectively. Results for each antibiotic and susceptibility results of bacterial populations tested are in table 1. Quality control results of control strains were within limits on both, MHA and MHChr.
**Conclusions:** RP includes inoculum standardisation in broth and chromogenic detection of mixed growth. If monomicrobial growth from urine sample occurs, AST results of RP are available one day before SP, an important advantage of RP.
Further studies are necessary to determine possible use and performances of RP in different circumstances.

TABLE 1. Comparative performances of standard procedure and rapid procedure in susceptibility testing of 200 isolates of *Enterobacteriaceae*.

| Antimicrobial | Procedure | No. of isolates | Test results in interpretative category, % | | | Performance analysis, % | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S | I | R | CA | mE | ME | VME |
| Ampicillin | Standard | 200 | 30.5 | 3.5 | 66 | | | | |
| | Rapid | 200 | 27 | 5 | 68 | 95 | 3.5 | 1.5 | 0 |
| Amoxicillin-clavulanic acid | Standard | 200 | 74.5 | 18.5 | 7 | | | | 0 |
| | Rapid | 200 | 72.5 | 19.5 | 8 | 91 | 9 | 0 | 0 |
| Piperacillin-tazobactam | Standard | 200 | 97 | 3 | 0 | | | | 0 |
| | Rapid | 200 | 92.5 | 6 | 1.5 | 94.5 | 5 | 0.5 | 0 |
| Cefuroxime | Standard | 200 | 80.5 | 2 | 17.5 | | | | 0 |
| | Rapid | 200 | 75.5 | 6.5 | 18 | 93.5 | 6.5 | 0 | 0 |
| Cefixime | Standard | 200 | 85 | 0.5 | 14.5 | | | | 0 |
| | Rapid | 200 | 83.5 | 2 | 14.5 | 96.5 | 2.5 | 0.5 | 0.5 |
| Ceftriaxone | Standard | 200 | 85 | 2.5 | 12.5 | | | | 0 |
| | Rapid | 200 | 84.5 | 1.5 | 14 | 98.5 | 1 | 0.5 | 0 |
| Ceftazidime | Standard | 200 | 89.5 | 2.5 | 8 | | | | 0 |
| | Rapid | 200 | 88 | 2.5 | 9.5 | 96 | 3 | 1 | 0 |
| Cefepime | Standard | 200 | 94.5 | 2 | 3.5 | | | | 0 |
| | Rapid | 200 | 93.5 | 2.5 | 4 | 98.5 | 1.5 | 0 | 0 |
| Imipenem | Standard | 200 | 100 | 0 | 0 | | | | 0 |
| | Rapid | 200 | 100 | 0 | 0 | 100 | 0 | 0 | 0 |
| Meropenem | Standard | 200 | 100 | 0 | 0 | | | | 0 |
| | Rapid | 200 | 100 | 0 | 0 | 100 | 0 | 0 | 0 |
| Gentamicin | Standard | 200 | 84.5 | 0 | 15.5 | | | | 0 |
| | Rapid | 200 | 83.5 | 0 | 16.5 | 99 | 0 | 1 | 0 |
| Amikacin | Standard | 200 | 99 | 1 | 0 | | | | 0 |
| | Rapid | 200 | 99.5 | 0.5 | 0 | 99.5 | 0.5 | 0 | 0 |
| Co-trimoxazole | Standard | 200 | 56 | 0 | 44 | | | | 0 |
| | Rapid | 200 | 55.5 | 0.5 | 44 | 97.5 | 0.5 | 1 | 1 |
| Ciprofloxacin | Standard | 200 | 67 | 2.5 | 30.5 | | | | 0 |
| | Rapid | 200 | 66 | 2.5 | 31.5 | 98 | 2 | 0 | 0 |
| Nitrofurantoin | Standard | 200 | 79.5 | 7 | 13.5 | | | | 0 |
| | Rapid | 200 | 80.5 | 6.5 | 13 | 92.5 | 6.5 | 0 | 1 |

Abbreviations: S, susceptible; I, intermediate; R, resistant; CA, categorical agreement; mE, minor error; ME, major error; VME, very major error.

## Objective

With rising rates of resistance, empiric treatment is less reliable than in the past and rapid results of antimicrobial susceptibility testing (AST) (time from the sample to the result) are very important.

The aim of this study was to evaluate the reliability of a new rapid one-day procedure (RP) by comparing AST results of RP to results of the standard two-day procedure (SP) for processing urine samples.

## Materials & Methods

Routine urine samples were processed by SP and RP and results of AST for 15 antimicrobial agents were compared.

List of antimicrobial agents studied: ampicillin (AM), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), cefuroxime (CXM), cefixime (CFM), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), imipenem (IPM), meropenem (MEM), gentamicin (GM), amikacin (AN), co-trimoxazole (SXT), ciprofloxacin (CIP), nitrofurantoin (FM).

SP: Urine was cultivated on chromogenic Uriselect 4 agar (Biorad, France) and isolated colonies were tested next day by standard CLSI disk-diffusion. Mueller Hinton agar (MHA - Biolife, Italy) and antimicrobial agent disks (Becton Dickinson, USA) were used. Rapid methods or Vitek 2 (bioMerieux, France) were used for identification of isolates.

RP: Urine (0.5 mL) was inoculated into eugonic broth vials and incubated in HB&L Uroquattro (both Alifax, Italy). Positive samples were incubated to turbidity of at least Mc Farland 2.0 (3 to 6 hours) and Gram stained. If only Gram-negative rods were seen, suspension was diluted to McFarland 0.5 (Densicheck, bioMerieux, France) and used as inoculum for CLSI disk-diffusion method with two modifications; different agar and different reading of plates was used:

- Chromatic Mueller Hinton agar (MHChr) (Liofilchem, Italy) was used instead of MHA. On this agar, different species grow with different colours.
- Inhibition zones of mauve colonies on MHChr were measured against a white background (mauve colour diffuses from colonies into agar and the border of colony growth was not visible well against black background). Zones of inhibition of blue and white-brown colonies were read against a black background according to CLSI guidelines.

Only results of monomicrobial growth of *Enterobacteriaceae* were considered in this study; if growth of few probable enterococcal colonies (small blue - turquoise colonies) occurred between highly predominant growth of Enterobacteriaceae on Uriselect 4 agar and on MHChr, it was neglected (and growth considered as monomicrobial).
For quality control ATCC strains *Escherichia coli 35218* (for AMC and TZP) *and E. coli 25922 (*for all other antimicrobial agents) were used. The same inoculum was used for control of MHA and MHChr.

Definitions:
Differences between AST results of SP and RP were classified as minor errors (one result intermediate, others not), major errors (false resistance in RP) and very major errors (false susceptibility in RP). All parameters were expressed as percentage among all isolates.

For targeted antimicrobial treatment, antimicrobial agents with "susceptible" result of RP AST would probably be used, so reliability of this result is crucial. True susceptibility results of RP were calculated. Definition of true susceptibility is: probability, expressed in percentage, that "susceptible" result of RP is correct result, confirmed by SP.

## Results (1)

**Table 1: Species of 200 *Enterobacteriaceae* isolates, isolated in the study.**

| Isolate (species) | Number of isolates | Proportion (%) |
|---|---|---|
| *Escherichia coli* | 153 | 76.5 |
| *Klebsiella pneumoniae* | 19 | 9.5 |
| *Proteus mirabilis* | 15 | 7.5 |
| *Citrobacter koseri* | 3 | 1.5 |
| *Morganella morganii* | 3 | 1.5 |
| *Citrobacter freundii* | 2 | 1 |
| *Proteus vulgaris* | 2 | 1 |
| *Enterobacter aerogenes* | 1 | 0.5 |
| *Klebsiella oxytoca* | 1 | 0.5 |
| *Providencia stuartii* | 1 | 0.5 |

**Performance analysis**

AST results of RP, compared to SP: from 3000 antimicrobial agent results, 2900 results (96.7%) were in complete agreement (CA).

Rates of minor errors (mE), major errors (MA) and very major errors (VME) were 2.8%, 0.4% and 0.2%, respectively. Results for each antimicrobial agent are in table 2.

**TABLE 2. Antimicrobial susceptibility testing of 200 isolates of *Enterobacteriaceae* - performance analysis of rapid procedure, compared to standard procedure.**
Abbreviations: CA, categorical agreement; mE, minor error; ME, major error; VME, very major error.

| Antimicrobial agent | Performance analysis, % | | | |
|---|---|---|---|---|
| | CA | mE | ME | VME |
| Ampicillin | 95 | 3.5 | 1.5 | 0 |
| Amoxicillin-clavulanic acid | 91 | 9 | 0 | 0 |
| Piperacillin-tazobactam | 94.5 | 5 | 0.5 | 0 |
| Cefuroxime | 93.5 | 6.5 | 0 | 0 |
| Cefixime | 96.5 | 2.5 | 0.5 | 0.5 |
| Ceftriaxone | 98.5 | 1 | 0.5 | 0 |
| Ceftazidime | 96 | 3 | 1 | 0 |
| Cefepime | 98.5 | 1.5 | 0 | 0 |
| Imipenem | 100 | 0 | 0 | 0 |
| Meropenem | 100 | 0 | 0 | 0 |
| Gentamicin | 99 | 0 | 1 | 0 |
| Amikacin | 99.5 | 0.5 | 0 | 0 |
| Co-trimoxazole | 97.5 | 0.5 | 1 | 1 |
| Ciprofloxacin | 98 | 2 | 0 | 0 |
| Nitrofurantoin | 92.5 | 6.5 | 0 | 1 |

## Results (2)

**TABLE 4. Two hundred isolates of *Enterobacteriaceae* - comparative results of cumulative antibiogram obtained by standard and rapid procedure.**
Abbreviations: S, susceptible; I, intermediate; R, resistant.

| Antimicrobial agent | Procedure | % S | % I | % R | Difference - (%S SP) – (%S RP) |
|---|---|---|---|---|---|
| Ampicillin | Standard | 30.5 | 3.5 | 66 | |
| | Rapid | 27 | 5 | 68 | 3.5 |
| Amoxicillin-clavulanic acid | Standard | 74.5 | 18.5 | 7 | |
| | Rapid | 72.5 | 19.5 | 8 | 2 |
| Piperacillin-tazobactam | Standard | 97 | 3 | 0 | |
| | Rapid | 92.5 | 6 | 1.5 | 4.5 |
| Cefuroxime | Standard | 80.5 | 2 | 17.5 | |
| | Rapid | 75.5 | 6.5 | 18 | 5 |
| Cefixime | Standard | 85 | 0.5 | 14.5 | |
| | Rapid | 83.5 | 2 | 14.5 | 1.5 |
| Ceftriaxone | Standard | 85 | 2.5 | 12.5 | |
| | Rapid | 84.5 | 1.5 | 14 | 0.5 |
| Ceftazidime | Standard | 89.5 | 2.5 | 8 | |
| | Rapid | 88 | 2.5 | 9.5 | 1.5 |
| Cefepime | Standard | 94.5 | 2 | 3.5 | |
| | Rapid | 93.5 | 2.5 | 4 | 1 |
| Imipenem | Standard | 100 | 0 | 0 | |
| | Rapid | 100 | 0 | 0 | 0 |
| Meropenem | Standard | 100 | 0 | 0 | |
| | Rapid | 100 | 0 | 0 | 0 |
| Gentamicin | Standard | 84.5 | 0 | 15.5 | |
| | Rapid | 83.5 | 0 | 16.5 | 1 |
| Amikacin | Standard | 99 | 1 | 0 | |
| | Rapid | 99.5 | 0.5 | 0 | -0.5 |
| Co-trimoxazole | Standard | 56 | 0 | 44 | |
| | Rapid | 55.5 | 0.5 | 44 | 0.5 |
| Ciprofloxacin | Standard | 67 | 2.5 | 30.5 | |
| | Rapid | 66 | 2.5 | 31.5 | 1 |
| Nitrofurantoin | Standard | 79.5 | 7 | 13.5 | |
| | Rapid | 80.5 | 6.5 | 13 | -1 |

## Conclusions

1. RP includes inoculum standardisation in broth, normal time of incubation of AST and chromogenic detection of mixed or pure growth on MHChr.
2. AST results of RP are available one day before SP, an important advantage of RP, especially when prevalence of resistance is high.
3. Rates of ME and VME were low for all antimicrobial agents; rates of mE were dependent on antimicrobial agent tested.
4. Rates of true susceptibily of RP "susceptible" result were high (96.3% - 100%; for eight antimicrobial agents percentage of true susceptibility was 100%).
5. Further studies are necessary to determine rational use and performances of RP in different circumstances.

**Table 3: Number of susceptible results of rapid procedure among 200 *Enterobacteriaceae* and percentage of true susceptibility among susceptible results of RP.**

| Antimicrobial agent | FM | AMC | AM | SXT | CFM | CAZ | AN | GM | CIP | TZP | CXM | CRO | FEP | MEM | IPM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of susceptible isolates of RP | 161 | 145 | 54 | 111 | 167 | 176 | 199 | 167 | 132 | 185 | 151 | 169 | 187 | 200 | 200 |
| Percentage of true susceptibily (%) | 96.3 | 96.6 | 98.1 | 98.2 | 98.8 | 99.4 | 99.5 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

***REFERENCES***
1. *Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.*
2. *Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 22nd informational supplement M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.*

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