Validation of Susceptibility Testing Results from Organisms Isolated Directly from Colorex Orientation® Agar

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ABSTRACT

Objectives: Chromogenic media offer the potential for more rapid identification of urinary pathogens while improving workflow by rapid elimination of contaminated culture. In 2012, an evaluation of multiple chromagars determined that Colorex Orientation Agar (CLX: Alere) provided the most accurate and reliable results when compared to standard urine culture (SUC). In order to incorporate the CLX into the lab workflow, this study was performed to validate the accuracy of susceptibility results of colonies taken directly from CLX.. **Methods:** A total of 119 organisms (67 Enterobacteriaceae [ENT], 45 Gram positive cocci [GPC] and 7 *Pseudomonas aeruginosa* [PA]) were sub-cultured onto CLX and set up directly to Vitek 2® (bioMérieux) automated susceptibility testing (AST) panels and recorded as susceptible (S), intermediate (I) or resistant (R). Each AST result was compared to the SUC AST result and categorized as total agreement (TA), minor disagreement-mD (no change in report), major disagreement-MD (change in report, not clinically significant), and very major disagreement- VMD (clinically significant change in report). **Results:** Of 1266 different antibiotic (AB) combinations tested for ENT, 12mD and 3MD were detected for a TA of 98.8%. For GPC, there were 765 AB combinations tested, and 2mD and 1VMD were seen giving a TA of 99.6%. For PA there were 125 AB combinations with 100% TA. Overall TA for all AB/organism combinations in this study was 99.2%. **Conclusions:** Based on these results, susceptibility testing can be performed accurately and reliably directly from colonies grown on CLX. Incorporation of CLX for work-up of urinary tract pathogens is anticipated to improve turn-around time to reporting of both identification and susceptibility results.

INTRODUCTION AND OBJECTIVES

Urine cultures are a common and time-consuming test in microbiology laboratories. Processing urine cultures requires multiple steps, including inoculation of multiple media, and interpretation of colony counts in addition to manual and automated identification tests. Chromagar plates for urine samples have been developed to isolate, enumerate and directly identify the most common uropathogens associated with community acquired urinary tract infections, including *E.coli, Proteus spp , Enterococci spp, S.saprophyticus, P.aeruginosa, S. aureus, C.albicans* as well as the Klebsiella , Enterobacter, Serratia and Citrobacter group (KESC). The plates can be inoculated directly from a urine sample for rapid detection, isolation and identification. In 2012, an Interior Health study was done to compare three such plates in terms of accuracy and reliability of identification results to routine urine culture methods. From this study, Colorex Orientation Agar® (CLX; Alere) was determined to be the best fit, in terms of accuracy and reliability of identification compared with standard culture.

The purpose of this study was to validate CLX to determine if automated susceptibility testing using the Vitek II® (bioMeriéux) system could be reliably interpreted directly from the chromogenic media.

Interior Health

METHODS

Previously characterized organisms

49 bacterial isolates (1 *Citrobacter amalonaticus*, 1 *Citrobacter freundii*, 2 *Enterobacter cloacae*, 6 *Enterococcus spp.*, 17 *Escherichia coli*, 1 *Proteus mirabilis*, 2 *Pseudomans aeruginosa*, 15 *Staphylococcus aureus*, 4 *Staphylococcus epidermidis*) with confirmed mechanisms of antimicrobial resistance were sub-cultured twice from frozen on Trypticase Soy Agar (TSA). Once purified, colonies were inoculated on (1) a CLX plate and (2) a TSA plate (standard urine culture media- SUC) for colony isolation and incubated at 35ºC for 18-20 hours. A 0.5 McFarland solution was made for each plate, and set up on the Vitek II® XL Automated Susceptibility Testing (AST) instrument. Results were recorded and compared for both SUC and CLX. Discrepancies were repeated

Clinical Samples

70 consecutive urinary tract pathogens (2 *C.freundii*, 7 *Enterococcus spp.*, 1 *E.cloacae*, 30 *E.coli*, 1 *Klebsiella oxytoca*, 6 *Klebsiella pneumoniae*, 5 *P.mirabilis*, 5 *P.aeruginosa*, 6 *S.aureus*, 1 *S.epidermidis*, 1 *Staphylococcus haemolyticus*, 1 *Staphylococcus hominis*, 2 *Staphylococcus saprophyticus*, 2 *Streptococcus agalactiae*) were isolated from clinical urine samples and set up on SUC and CLX and tested by the Vitek II® XL AST system. Results were recorded and compared. All susceptibility results from the CLX and SUC were categorized as a Total Agreement (TA- No category change, within one MIC dilution,) minor disagreement (mD- Susceptible[S] -Intermediate[I], I-S, I- Resistant[R], R-I), major disagreement (MD- CLX=R, SUC=S) and very major disagreement (VMD- CLX=S, SUC=R).

RESULTS

Enterobacteriaciae

Of the 119 organisms tested, 67 were Enterobacteriaceae (ENT). All 67 isolates were set up on Vitek AST-N213 cards for a total of 1266 Antibiotic(AB)/ENT combinations.

Out of the 1266 combinations, there were 12mD and 3MD for a TA of 98.8% (1251/1266) (Table 1).

Table 1. Agreement between 67 tested ENT and the respective antibiotics on the Vitek AST-N213 card. Results are shown as CLX/SUC.

Organism	n	ENT/AB combinations	mD	MD	VMD	TA
C.amalonaticus	1	19	0	0	0	100%
C.freundii	3	57	0	0	0	100%
E.cloacae	3	57	(1)- Nitrofurantoin (I/S)	(1)- Ertapenem (R/S)	0	96%
E.coli	47	893	(9)- Amox/Clav (I/S) Amox/Clav (R/I) Cephalothin (I/S) x2 Cefazolin Urine Cefazolin Other Cefoxitin (R/I) Cefoxitin (S/I) Nitrofurantoin (S/I)	(1)- PipTazo (R/S)	0	99%
K.oxytoca	1	19	0	0	0	100%
K.pneumoniae	6	114	(1)- PipTazo (I/S)	0	0	99%
P.mirabilis	6	114	(1)- Tobramycin (I/S)	(1)- Gentamicin (R/S)	0	98%

RESULTS

Gram Positive Cocci

Of the 119 organisms, 45 were Gram positive cocci (GPC). All 45 isolates were set up on Vitek AST-GP67 cards for a total of 765 AB/GPC combinations.

Of 765 combinations, there were 1 mD, 1VMD and 1 antibiotic terminated by the Vitek II XL and deemed a mE (TRM). This gave a final TA of 99.6% (Table 2).

Pseudomanas aeruginosa

Of the 119 organisms, 7 were *Pseudomonas aeruginosa* (PA). All 7 isolates were set up on the Vitek AST-N222 cards for a total of 119 AB/PA combinations.

Of the 119 combinations, no errors were seen, for a final TA of 100%.

A total of 2150 AB/organism combinations were tested in this study, for a final total agreement of 99.2% (2133/2150)

Table 2. Agreement between 45 tested GPC, and the respective antibiotics on the Vitek AST-GP67 card. Results are shown as CLX/SUC.

Organism	n	GPC/AB combinations	mD	MD	VMD	TA
Enterococcus spp.	13	195	(1)- Erythromycin (I/R)	0	0	99%
S.aureus	21	378	0	0	0	100%
S.epidermidis	5	90	0	0	(1)- Tetracycline (S/R)	99%
S.haemolyticus	1	18	0	0	0	100%
S.hominis	1	18	(1)- Tigecycline (TRM)	0	0	94%
S.saprophyticus	2	36	0	0	0	100%
S.agalactiae	2	30	0	0	0	100%

DISCUSSION

Overall, there was excellent correlation between CLX and SUC media on all three Vitek cards (AST-N213, AST-GP67,AST-N222)

Discrepencies would not have had clinical impact in the following cases:

- Any Pip/Tazo resistance not associated with ESBL or fully expressed AmpC cephalosporinase is routinely confirmed by an alternate method. The CLX was considered correct as the *E.coli* strain expressed an AmpC cephalosporinase (confirmed by MAST® disc test and Vitek)
- Ertapenem resistance is always confirmed with supplemental testing.
- Erythromycin is not reported on urine isolates, so the discrepancy would have had no clinical impact.

This study found an excellent categorical agreement (100%) for *Pseudomonas aeruginosa* isolates. However a similar study in 2012 (Turnbull & Rennie- CACMID 2013) demonstrated a lower categorical agreement for this organism. Both studies involved a small sample size (n=7 IH study, n=10 Turnbull and Rennie), so further validation should be considered of direct testing from CLX for *P.aeruginosa*.

CONCLUSION

Based on the study results, susceptibility testing can be reliably performed directly from the Colorex Orientation Agar® on Vitek II® using AST-N213, AST-GP67 and AST-N222 cards.