Comparison of three selective media for the recovery of Extended Spectrum β-Lactamase (ESBL)-producing *Enterobacteriaceae*

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REVISED ABSTRACT

Background: Over the past two years, an increasing number of ESBL-producing organisms have been recovered from our patient population. ESBL-producing organisms are particularly problematic in our bone marrow transplant population since empiric sepsis therapy in febrile neutropenic patients may not cover these organisms.

Methods: We compared the recovery of ESBL-producing organisms from rectal swabs submitted for VRE screening on three media: vancomycin, amphotericin B, ceftazidime, and clindamycin (VACC) agar (Remel, Lenexa, KS), MacConkey, ceftazidime, cloxacillin (MCC) agar, and CHROMagar (CA-ESBL) agar (Lexington, KY). After inoculation of VRE screening plates, the swab content from 566 specimens was suspended in 500 ul of sterile saline and 100 ul was inoculated onto each plate. Plates were examined at 24 and 48 hours. Plates from which Gram-negative bacilli (GNB) were recovered were screened for ESBL production using ceftazidime (CZ), cefotaxime (CX), CZ-clavulanate (CA), and CX-CA disc diffusion, ampC production using cefotetan, cefotetan-cloxacillin Etests, and the modified Hodge test following ertapenem screening.

Results: No growth for all three media was observed for 355 specimens. Growth was observed on at least one medium for 211 specimens. ESBL-producing organisms were recovered from only 26 (4.5%) patient specimens. VACC was the most sensitive at 92%, MCC at 85% and CA-ESBL at 81%. Unfortunately none of the media was specific; ampC-producing organisms were found in 27 specimens (4.7%): with 25 isolates growing on VACC, 19 on CA-ESBL and 6 on MCC. Three Hodge test positive isolates were recovered on all three media. Other MDR-GNBs were recovered as well with 45 positive cultures on CA-ESBL, 42 on VACC, and 32 on MCC. Additionally Gram-positive organisms grew on 86 VACC, 4 MCC, and 11 CA-ESBL. Break through growth was seen predominantly after 48 hr especially with gram positives so limiting incubation to 24 hours would greatly improve specificity and positive predictive value.

Conclusions: Recovery of ESBL-producing organisms was similar on all three media.. Screening of rectal swab for ESBL-producing organisms is very labor intensive due to the poor specificity of currently available media.

INTRODUCTION

Multi-drug resistant (MDR) gram negative bacilli (GNB) are becoming an increasing clinical challenge. MDR-GNB often produce beta-lactamase enzymes that fall into three broad categories:

1. extended spectrum beta-lactamases (ESBL)
2. ampC beta-lactamase hyper-producers (ampC)
3. carbapenemases

Several factors have resulted in our having heightened interest in beta-lactamase producing MDR-GNB:

1. An increase in the number of ESBL-producing *Enterobacteriaceae* clinical isolates from 1.9% in 2009 to 3.2% in 2010 at our institution.
2. The recognition of the rapid global spread of "new" beta-lactamases such as NDM. VIM, and KPC. Treatment options for organisms with these resistance mechanisms are very limited, colistin or tigecycline. Because of this, it has been recommended that fecal/rectal screening for these organisms be undertaken in exposed individuals although no guidance has been offered on how this might best be done.(1)
3. The recognition that practice guidelines for empiric therapy of high-risk neutropenic patients currently does not recommend agents active against ESBL-producing organism except in clinical situations where ESBL status is known (2). We recently had a high risk neutropenic patient treated with cefepime and vancomycin who developed septic shock and died. He was found to have bacteremia due to an ESBL-producing *Escherichia coli* strain. As a result our hematologists asked us to examine the problem of ESBL-producing organism colonization in our BMTU.
4. Currently there is no recommended method for the detection of fecal carriage of ESBL-producing organisms.

This study has three goals.

1. Determine which of three media, vancomycin, amphoteracin B, ceftazidime and clindamycin (VACC) blood agar (Remel, Lenexa Ks), MacConkey cloxacillin ceftazidime (MCC) agar and CHROMAgar ESBL (CA-ESBL) agar (Gibson Laboratories Lexington, KY) is has the best performance at recovering ESBLs from rectal swabs.
2. Determine the frequency with which ESBL-producing organisms are found in the general hospitalized patient population our institution.
3. Determine the frequency with which ESBL-producing organisms are recovered from rectal swabs of patients in our Bone Marrow Transplant Unit.

RESULTS

Table 1: Growth on VACC, MCC and CA-ESBL after 48-hours incubation

Organism type isolated	VACC	MCC	CA-ESBL
ESBL (n = 26)	24	22	21
H AmpC (n = 27)	25	6	19
carbapenemase (n = 3)	3	3	3
gnr	42	32	45
gpc	79	0	0
Yeast	18	4	11
No growth	375	499	467

Table 2: ESBL isolates detected

Escherichia coli	13*
Entrobacter species	6*
Klebsiella species	4*
Hafnia species	2
Citrobacter species	1
Raoultella species	1
Serratia species	1

*One specimen grew ESBL-producing strains of E. coli and Enterobacter aerogenes
One specimen grew ESBL-producing strains of E. coli and Klebsiella pneumoniae

Table 3A: Performance of VACC, MCC, CA-ESBL for detection of ESBL: any growth

	VACC	MCC	CA-ESBL
Sensitivity	92	85	81
Specificity	69	91	86
PPV	12	31	21
NPV	99	99	99

Table 3B: Performance of VACC, MCC, CA-ESBL for detection of ESBL: gram-negative growth only

	VACC	MCC	CA-ESBL
Sensitivity	92	85	81
Specificity	85	92	87
PPV	23	33	23
NPV	99	99	99

Table 4: ESBL detection in BMTU specimens on VACC, MCC, CA-ESBL 48-hours incubation

Organism type isolated	VACC	MCC	CA-ESBL
ESBL	2*	2	2
H AmpC	1	1	1
carbapenemase	0	0	0
gnr other	3	3	4
gpc	8	0	0
Yeast	0	0	0
No growth	97	102	101

*Both isolates recovered from same patient

CONCLUSIONS

1. ESBL-producing organisms were found in 4.5% of 566 fecal samples cultured on the three test media.
2. VACC plates were the most sensitive for the detection of ESBL-producing organisms @ 92%; however, the positive predictive value of ESBL detection when a gram negative bacilli grew on this medium was on 23%.
3. MCC had a better specificity with a positive predictive value of 33% in part because fewer hyper ampC-producing organisms were found on this medium (6/27 vs 25/27 for VACC).
4. CA-ESBL was the least sensitive medium @ 81%; its positive predictive value of growth of a gram negative organism on this medium was only 23% making it the weakest performing of the three media evaluated for detection of ESBL-producing organisms.
5. All three carbapenemase producing organisms were detected on all three media.
6. Approximately 1/5 of the specimens studied came from our BMTU. The number of ESBL-producers (N=2) was too low to warrant statistical analysis. Both isolates came from a single patient.
7. We concluded that routine screening of neutropenic bone marrow transplant patients for ESBL-producing organisms would be extremely labor intensive using available media given their poor positive predictive value. This observation is consistent with those of others using other selective media. (3-5) Additionally low detection rates in our BMTU makes this practice of questionable benefit for our BMT patients especially since fecal carriage of ESBL-producing. organisms has not been associated with infection due to those organisms(6). For now, we have abandoned this practice.

MATERIALS AND METHODS

1. Specimens submitted for vancomycin resistant enterococci screening were plated on appropriate medium and the swab was then placed in 500 ul of saline. The remaining specimen was suspended and 100 ul was inoculated onto each of three plates, vancomycin, amphotericin B, ceftazidime, and clindamycin (VACC) agar (Remel, Lenexa, KS), MacConkey, ceftazidime (4 ug/ml), cloxacillin (200 ug/ml) (MCC) agar, and CHROMagar-ESBL(CA-ESBL) agar (Gibson Laboratories, Lexington, KY). Specimens submitted for vancomycin resistant enterococci screening were plated on appropriate medium and the swab was then placed in 500 ul of saline. The remaining specimen was suspended and 100 ul was inoculated onto each of the three plates.
2. Plates were incubated @ 35°C and examined at 24 and 48 hours. Plates from which Gram-negative bacilli (GNB) were recovered were screened for ESBL production using ceftazidime (CZ), cefotaxime (CX), CZ-clavulanate (CA), and CX-CA disc diffusion, ampC hyperproduction using cefotetan, cefotetan-cloxacillin Etests, and the modified Hodge test following ertapenem screening. Growth of yeast and gram positive cocci was based on colonial morphology and gram stain. All ESBL-producing strains were speciated using standard laboratory methods.
3. This study was approved by the Biomedical IRB of the University of North Carolina Testing

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