Addition of disk diffusion based screening on chromogenic Mueller-Hinton agar adds value to traditional cephalosporin based screening for multiresistant Gram-negative bacteria

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Introduction and purpose

Screening for carriage of ESBL and carbapenemase producing Gram-negatives (CPG) is an increasing challenge. OXA-48 like enzymes puts the sensitivity of CPG screening methods to the test and poor specificity is a problem in low prevalent settings. We examined the added value of disk diffusion based screening using EUCAST zone diameter breakpoints and chromogenic Mueller-Hinton agar supplemented with vancomycin, in addition to a cephalosporin based screening plate for ESBLs.

Methods

Chromogenic Mueller-Hinton agar plates (CHROMagar MH Orientation) supplemented with 8 mg/L of vancomycin (CMH-V) were validated against Mueller-Hinton plates using the EUCAST standard.
Screening for multiresistant bacteria from 557 patients was done by complementing the standard plating onto CHROMagar ESBL plates by plating swabs (mainly rectal) onto CMH-V. Meropenem (10 µg), ceftazidime (10 µg), temocillin (30 µg) and piperacillin/tazobactam (30/6 µg) disks were applied before overnight incubation. Colonies on ESBL plates or within the anticipated susceptibility diameters defined by EUCAST on CMH-V were subject to species identification and susceptibility testing, as were 83 randomly chosen, apparently susceptible isolates growing on CMH-V only.

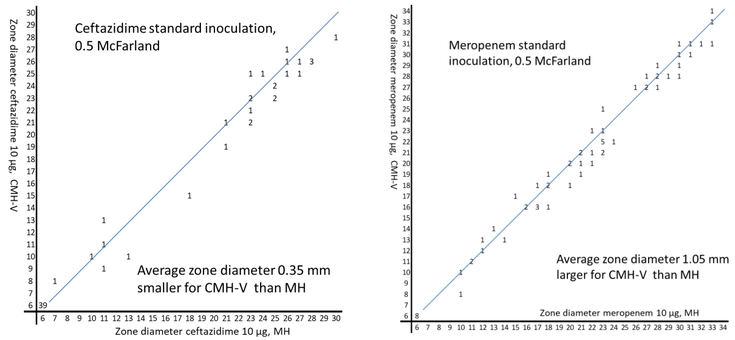

Figure 1. Inhibition zone diameter comparison for ceftazidime and meropenem of 0.5 McFarland inoculates of Gram-negative bacteria plated on chromogenic and standard Mueller-Hinton plates, respectively. The blue line represents identical zone diameters.

Table 1. Performance of CMH-V screening with disk diffusion for ceftazidime, meropenem, piperacillin/tazobactam and temocillin compared to cephalosporin-based screening on ESBL-plates

Number of patients:	***n***	**Percentage**
screened for ESBL	557	
with no growth on CMH-V	127	22.8 %
with growth on CMH-V	430	77.2 %
with growth of non-relevant species on CMH-V	36	6.5 %
with growth on ESBL-plate	81	14.5 %

Number of isolates that grew on:	***n***	**Percentage**
CMH-V	506	
CMH-V but with non-relevant species (non ESBL/CPG)	39	7.7 %
ESBL-plate	89	17.6 %
ESBL-plate but with non-relevant species (non ESBL/CPG)	10	2.0 %
ESBL-plate with species harbouring intrinsic AmpC	8	9.0 %

Benefits of CMH-V:	***n***	**Percentage**
Overall benefit	18	3.2 %
Relevant isolates on ESBL-plates that could be ruled out as CPGs	11	13.9 %
Intrinsic AmpC-producers on ESBL plates ruled out as CPGs	7	
Identified multi-resistant isolate not detected on ESBL-plate	6	1.2 %
Carbapenem producing *Klebsiella pneumoniae* (OXA-48)	1	
Carbapenem producing *Escherichia coli* (OXA-181)	1	
ESBL producing *Salmonella enterica*	1	
AmpC producing *E. coli*	2	
Vancomycin resistant *Enterococcus faecium*	1	

Limitations of CMH-V:	***n***	**Percentage**
Too sparse growth on CMH-V to read inhibition zones	54	10.7 %
Non-complete CMH-V screening due to loss of disks	3	0.7 %

Results

Zone diameters on CMH-V and MH correlated well with a <0.5 mm average difference for all tested antibiotics (Figure 1).

The performance of the direct disk diffusion based screening on CMH-V as a complement to screening Chromagar ESBL is outlined in Table 1 and exemplified in Figure 2. Among 89 isolates on ESBL plates, 79 consisted of species potentially harbouring ESBLs, plasmid-mediated AmpC or carbapenemases. Susceptibility testing proved all 60 *Escherichia coli* and 6 *Klebsiella pneumoniae* from the ESBL-plates as having ESBLs and/or AmpC, while 12 of the 13 remaining isolates, consisting of 9 naturally AmpC producing Enterobacterales, 3 *Pseudomonas aeruginosa* and 1 *Klebsiella oxytoca,* could be ruled out as CPGs. These 12 isolates could also have been ruled out as CPGs already from zone diameters on CMH-V utilizing the EUCAST algorithm for detection of carbapenemases with disk diffusion.

Reading of zone diameters on CMH-V enabled detection of 2 additional AmpC producing *E. coli*, 1 *Salmonella enterica* with ESBL, and two CPGs; 1 OXA-181 positive *E. coli* and 1 OXA-48 positive *K. pneumoniae* that would have remained undetected, using only ESBL-plates for screening.

Conclusions

Disk diffusion based screening using CMH-V agar enabled detection of CPGs and ESBL-producing bacteria otherwise not found and added both sensitivity and specificity to traditional ESBL plate-based screening.

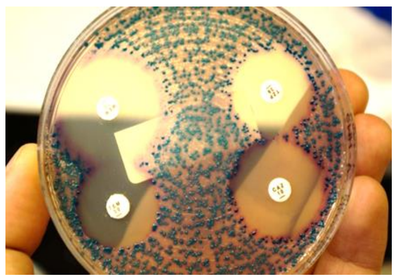

Figure 2. Example of screening showing susceptible *E. coli* and *K. pneumoniae* isolates.

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