Development, optimization, standardization and validation of a simple in-house agar gradient method to determine vancomycin MIC's for Staphylococcus aureus

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Background: MIC determination has become easier, with introduction of the E-test. The point of contention has been the cost of the commercial strips which has restricted its use in resource limited countries. We attempted to develop, standardize and validate a simple in – house agar gradient method, to determine MIC of vancomycin for clinical isolates of S. aureus.

Methods & Materials: In – house strips were made from Whatmann filter paper no 1. The strips were impregnated with varying concentration of vancomycin solution so as to create an increasing antibiotic gradient along the strip. During the standardization step, MIC's of 90 clinical strains of S. aureus and ATCC 29213 were tested by the broth microdilution and commercial strip followed by the in – house strip. The results were kept blinded during the development stage. Variables for the preliminary considerations for optimization and standardization of the In – House Agar Gradient strip were considered to correct the outliers. This was followed by validation stage where MIC's of 90 different clinical strains of S. aureus and ATCC 29213 were determined by the in – house and results were kept blinded. This was followed by determination of MIC's by broth microdilution and commercial strips. An MIC reading of ± 1log2 dilution compared with broth microdilution was considered as an outlier.

Results: During the optimization and standardization stage there were 7/90 outliers in the clinical strains and no outliers seen with the ATCC 29213 control strain. Corrective action was performed by increasing precaution during the antibiotic solution impregnation stage. During the validation stage, only 4/90 outliers were observed in the clinical strains. The commercial strips had 29/90 among clinical and 15/30 outliers in the control strain during the prevalidation phase. The supplier was informed to maintain cold chain and during the validation phase the outliers for commercial strip were 18/90 and 4/30 in the clinical and control strain respectively.

Conclusion: Vancomycin sensitivity is reported as MIC. Expensive commercial strips can be replaced by in – house strips using this simple technique after validating it with a gold standard method like broth microdilution.

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Multidrug-resistant acinetobacter baumanii – plasmid-borne carbapenem and aminoglycoside co-resistance causing outbreak in Southwest Virginia

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Background: Multidrug-resistant (MDR) Gram-negative bacterial pathogens, often resistant to more than one class of antibiotics including carbapenems and aminoglycosides, pose serious threats in healthcare settings worldwide. A 2015 White House task force issued guidelines to combat antibiotic-resistant strains in the United States, which the Center for Diseases Center has termed “nightmare bacteria”. MDR pathogens cause at least two million illnesses and 23,000 deaths annually in the U.S. MDR A. baumanii (MDR-Ab) cause 10% of hospital-acquired infections (HAI) with 70% patient mortality. A mini-outbreak of carbapenem-resistant (CR) MDR-Ab (CR-MDR-Ab) occurred during a 2009-2010 H1N1 epidemic at Carilion Medical Center (CMC) in Virginia, U.S; three of the nine patients died. To develop effective strategies for prevention, control and treatment of MDR infections, we are performing whole genome analysis of the clinical isolates of CR-MDR-Ab.

Methods & Materials: To date, we have analyzed 68 Ab clinical isolates, including five CR-MDR-Ab outbreak strains. We have sequenced and analyzed whole genomes of the following MDR-Ab isolates: an isolate from outbreak patients (CMC-MDR-Ab); a carbapenem-sensitive isolate (CMC-MDR-Ab59); and a CR-MDR-Ab (CMC-MDR-Ab66) isolate from sporadic cases. The sequencing was performed on the PacBioRSII platform.

Results: The CR CMC-MDR-Ab strains were found to carry two plasmids, pCMCVTAb1 and pCMCVTAb2. The latter is conjugative type and carried two transposons: Tn2008-like, containing a beta-lactamase gene (blaOXA23) and conferring CR, and a TnaphA6 element causing aminoglycoside resistance and further reducing treatment choices. Their chromosomes carried five blal genes and an aphA1 gene. A PCR analysis based upon the resistant determinants showed that all outbreak isolates (100%) carried pCMCVTAb2 containing the two transposons. Fourteen of the remaining 63 isolates (22.22%) carried pCMCVTAb1 and of these, six (42.85%) carried Tn2008-like, and two (14.28%) carried TnaphA6. These differences are statistically significant using Fisher’s exact, two-tailed p = 0.0011, p = 0.0445 and p = 0.0018.

Conclusion: The results suggested that pCMCVTAb2 was responsible for the CR-MDR outbreak. A PCR analysis based on the antibiotic-resistant genes of pCMCVTAb2 could be used for rapid identification of CR-MDR-Ab strains, thereby helping to prevent the