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ePoster Viewing
Susceptibility testing methods

Detection of extended-spectrum beta-lactamases in AmpC-producing organisms – a comparison of phenotypic and molecular methods

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Background: Phenotypic susceptibility testing cannot always differentiate between cephalosporin resistance due to hyper-production of chromosomal beta lactamases (AmpC) and the presence of extended spectrum beta lactamases (ESBLs) among certain Enterobacteriaceae. Knowledge of underlying resistance mechanisms is important for infection control, surveillance and antibiotic stewardship purposes. This study describes the phenotypic and molecular characterisation of selected cephalosporin resistant Enterobacteriaceae at a tertiary hospital in Cape Town, South Africa, and evaluates the ability of an automated susceptibility testing platform to identify the underlying resistance mechanism.

Material/methods: 50 isolates of species harbouring the AmpC beta lactamase (27 *Enterobacter cloacae*, 9 *Morganella morganii*, 8 *Citrobacter freundii*, 3 *Serratia marcescens* and 3 *Enterobacter aerogenes)* were selected on the basis of resistance to 3rd generation cephalosporins as determined by the Vitek2® system. Susceptibility testing was repeated using disc diffusion as well as gradient diffusion MICs for ceftriaxone, cefepime and ceftazidime. MICs were interpreted using CLSI guidelines. Phenotypic detection of ESBLs was performed using the double disc diffusion test. PCR for CTX-M, SHV and TEM related ESBLs was performed on all isolates.

Results: . Comparing Vitek2® to gradient diffusion results for cefotaxime, there were 8 minor errors, 21 major errors (ME) and 1 very major error (VME). For cefepime the numbers were 12 minor, 1 ME and 13 VMEs. Cefotaxime and ceftazidime MICs were generally higher when tested with Vitek2 than gradient diffusion; the converse was true for cefepime. The double disc diffusion test showed evidence of an ESBL in 15 (30%) isolates while the Vitek2® advanced expert system (AES) inferred the presence of an ESBL in 33 (66%) isolates; in 14 of these the AES could not differentiate between ESBL and derepressed AmpC as shown in Table 1. At least one ESBL gene was detected in 27 (54%) of the isolates by PCR, with 21 of the 27 containing 2 ESBL genes, and 3 containing all 3 genes. CTX-M was the commonest ESBL, found either alone or in combination in 20 isolates.

Conclusions: Discrepancies between susceptibility results using Vitek2® and gradient diffusion are common. Of particular concern is the possible undercalling of resistance to cefepime by Vitek2®; agar dilution MICs will be performed to further interrogate this. There is poor correlation between the Vitek2® AES and molecular presence of ESBLs, which may have implications for both infection control and antimicrobial therapy. Over half the isolates contained at least one ESBL gene; the basis

of extended spectrum cephalosporin resistance in the other isolates is likely due to hyperproduction of AmpC.

Vitek2® phenotype	ESBL PCR		Total
	Positive	Negative	
ESBL	13	6	19
ESBL /ampC	4	10	14
ampC	10	7	17

Table 1: Correlation between ESBL detection by Vitek2® AES and PCR