Detection of Extended-Spectrum Beta-Lactamases in AmpC-Producing Organisms: A Comparison of Phenotypic and Molecular Methods.

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Introduction:

Cephalosporin resistance in Enterobacteriaceae is commonly due to extended spectrum beta-lactamases, although in certain genera Ambler class C chromosomal beta lactamases (AmpC) also play a role, especially if there is constitutive hyperexpression of *ampC*. [1]

Phenotypic susceptibility testing methods (including automated systems) may not reliably distinguish between resistance to cephalosporins mediated by ESBLs and hyperexpression of *ampC*.

Understanding the epidemiology of bacterial resistance mechanisms is a key component of addressing the burden of antimicrobial resistance (AMR).

Aim:

To perform different phenotypic susceptibility testing methods on a collection of organisms known to harbour chromosomal *ampC*, and to compare these results to molecular methods.

Methods:

50 non-duplicate clinical isolates (Table 1) obtained from the routine diagnostic laboratory at Tygerberg Hospital, Cape Town, South Africa, were chosen on the basis of resistance to cefotaxime or ceftazidime when tested on the Vitek2® automated system. The Vitek2 advanced expert system (AES) classified the underlying resistance mechanism as derepressed *ampC*, ESBL, or both (i.e. unable to differentiate). (Fig 1)

Organism	Number
Enterobacter cloacae	27
Enterobacter aerogenes	3
Morganella morganii	9
Citrobacter freundii	8
Serratia marcescens	3

Table 1: Isolates used in the study

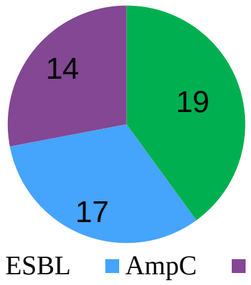
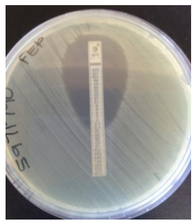


Fig 1: Cephalosporin resistance mechanism, as determined by Vitek AES.

Phenotypic susceptibility testing for cefotaxime, ceftazidime and cefepime was performed using disc diffusion and gradient diffusion MICs, and interpreted using CLSI guidelines. Vitek2 and disc diffusion results were compared to gradient diffusion MIC results (Figs 2a and 2b).

A 

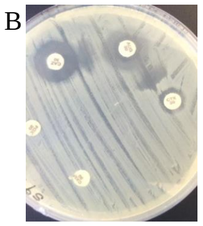
B 

Fig 2a: Gradient diffusion MIC
Fig 2b: Disc diffusion (showing ESBL activity using double disc diffusion test)

A multiplex PCR assay[2] for TEM, SHV and CTX-M ESBLs was performed on all isolates (Fig 3).

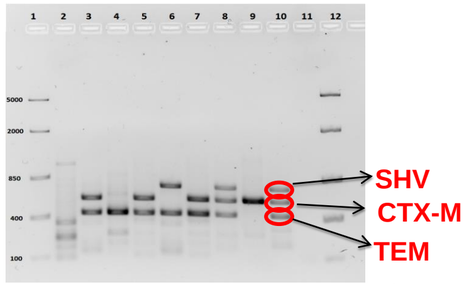


Fig 3: Agarose gel showing results of mPCR, highlighting appropriate amplification products

Results

Cefotaxime and ceftazidime MICs were generally higher when tested with Vitek 2 compared to gradient diffusion; the reverse was true for cefepime.

Error	Cefotaxime		Ceftazidime		Cefepime	
	Vitek	Disc	Vitek	Disc	Vitek	Disc
Minor	8	8	6	7	12	17
Major	21	21	9	7	1	0
Very Major	1	1	0	1	13	10

Table 2: Numbers of errors detected when comparing disc diffusion and Vitek2 results to gradient diffusion MIC.

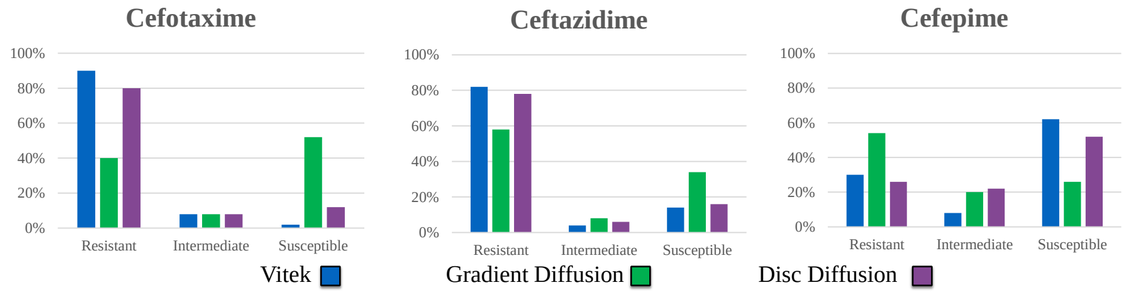


Fig 4: Distribution of susceptibility testing results for the three cephalosporins, using three different methods

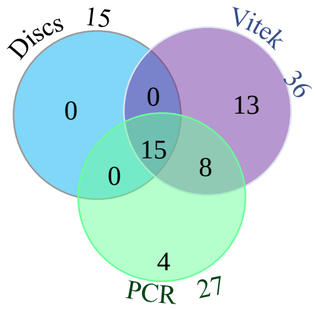


Fig 5: Correlation of ESBL detection by Vitek, disc diffusion and PCR.

Vitek AES mechanism	ESBL PCR Pos	ESBL PCR Neg
ESBL	13	6
ESBL or ampC	10	7
ampC	4	10

Table 3: Comparison of Vitek AES deduced mechanism with PCR results.

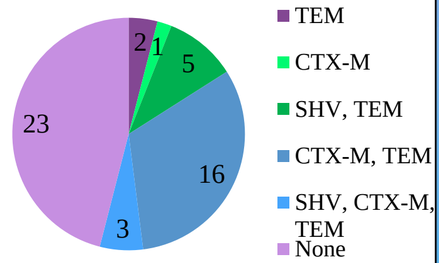


Fig 6: Results of PCR for TEM, SHV and CTX-M genes.

Discussion

Discrepancies between susceptibility results using Vitek2 and other MIC methods have been described, including high very major error rates for cefepime[3]. This is concerning given renewed interest in cefepime as an alternative for carbapenems[4]. Broth dilution MICs will be performed to serve as a better reference standard. There was a poor correlation between the Vitek2 AES deduced mechanism and molecular presence of ESBLs, which may have implications for both infection control and antimicrobial therapy. TEM-type genes were most commonly detected, followed by CTX-M, in line with previous work in South Africa[5]. However other studies have found SHV to be very common among local *Klebsiella pneumoniae*[6].

References

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