ECCMID 2015
Copenhagen

P0163

Laboratory evaluation of the GENSPEED® Superbug CR assay for the detection of carbapenemase genes in bacterial isolates.

N.Reading, H. Morton, N.Ratnaraja

Sandwell and West Birmingham Hospitals NHS Trust, Birmingham, United Kingdom.

Sandwell and West Birmingham Hospitals NHS
NHS Trust

Introduction

Differentiation of Carbapenem resistance in Gram negative bacteria mediated by expression of extended spectrum β-lactamase (ESBL) or AmpC production combined with efflux pumps and porin loss and resistance caused by carbapenemase production can be challenging.

To improve detection of isolates which carry carbapenemase genes, we evaluated the performance of a new research use only test, the GENSPEED® Superbug CR assay.

The GENSPEED® Test System combines a disposable microfluidic test chip with gene probes for blaVIM, blaNDM, blaOXA-48 and blaKPC and uses a chemi-luminescence-based device for the detection of PCR amplification products.

Method

A total of 89 isolates (K. pneumoniae [n=34], E. coli [n=24], P. aeruginosa [n=10], E. cloacae [n=8], A. baumanii [n=4], K. oxytoca [n=1], Salmonella spp [n=2], E. aerogenes [n=2], C. freundii [n=1], M. morganii [n=1] S. maltophilia [n=1] and S. marsecens [n=1], were tested for the presence of Carbapenemase genes including blaNDM [n=18], blaKPC [n=7], blaOXA-48 [n=11], blaIMP [n=7], blaOXA-23 [n=1] and blaVIM [n=4].

One isolate possessed both blaOXA-48 and blaNDM.

The rest had mechanisms contributing to carbapenem resistance: ESBL or AmpC plus porin loss [n=21], OprD mutation/efflux pumps [P. aeruginosa, n=5], wild type strains or miscellaneous mechanisms (but carbapenemase negative =) [n=14].

Method (Continued)

Isolates were cultured on Columbia Blood Agar (Oxoid, Basingstoke, U.K.) and prepared by inoculating 1µl of bacterial colony into a lysis buffer (solution A) (Greiner Bio-One, Stonehouse, U.K.) shaken with beads for 30 seconds and then 5 drops of lysate were collected in a collection tube (Figure 1).

20µl of lysate was then added to a lyophilized master-mix (solution B) provided in the GENSPEED Superbug CR assay (Greiner Bio-One, Stonehouse, U.K) and the PCR reaction was run on a thermocycler (Figure 2).

P.C.R. product was added to an hybridization buffer (Solution C), denatured at 95˚ C for 1 minute (Figure 3) and then applied onto the test chip (Figure 4).

Enzyme (Solution D), Wash Solution (Solution E) and Chemi-Luminescent substrate (Solution F) were added sequentially at the required time points (Figure 5).

Chemi-luminescence signals were read in the GENSPEED® Reader and quantified by the GENSPEED® Software (Figure 6).

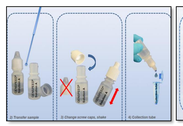

Figure 1. Prepare Sample

Figure 2. Perform PCR reaction

Figure 3. Denature PCR product

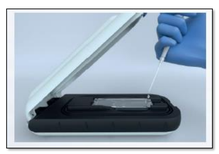

Figure 4. Hybridize on chip

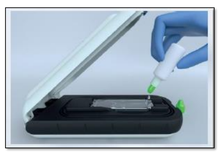

Figure 5. Add Chemi-Luminescence Reagents

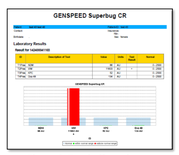

Figure 6. Read off results

Discrepant analysis was performed using the Checkpoints Check MDR CT103 XL microarray panel. (Checkpoints BV, Netherlands).

Results

When only included targets (KPC,NDM,VIM,OXA-48) are considered (n=81), the GENSPEED® Superbug CR assay performed as follows:

Table 1.

	Result	95% C.I. Limits
Sensitivity	97.56%	87.1 - 99.6
Specificity	100%	91.1 - 100
Negative Predictive Value	87.56%	87.1 - 99.6
Positive Predictive Value	100%	91.11 - 100

Table 2.

Results for each Carbapenemase Gene

Genotype	Number Detected	Percentage
KPC (n= 7)	7/7	100%
NDM (n= 18)	17/18	94.44%
VIM (n= 4)	3/4	75%
OXA-48 (n= 11)	11/11	100%
NDM & OXA 48 (n=1)	1/1	100%
IMP (n= 7)	0/7	0% (target not included in assay)

In our laboratory, prevalence of carbapenemase producers amongst carbapenem resistant isolates is estimated at 10%.

When this is considered the GENSPEED® Superbug CR assay's estimated P.P.V. is 100% and N.P.V. 99.7%.

When all tested organisms are considered (n=89), assay performance becomes:-

Sensitivity 81.63%, Specificity 100 %, N.P.V. 81.63%, P.P.V. 100%.

Conclusions

The GENSPEED® Superbug CR assay is a simple to use test, with only 5 minutes of hands on time and results available within 75 minutes.

The assay provides accurate results with an excellent positive and negative predictive when used routinely (PPV 100%, NPV 99.7%).

The assay may prove useful for use on colonies recovered from Carbapenem screening agar and on isolates known to be Carbapenem resistant from susceptibility testing.

Genotypes detected in the assay could be modified to include other genes as dictated by epidemiological considerations.

Acknowledgements

Many thanks to Dr. Mandy Wooton, Public Health Wales, Cardiff and to Dr. John Perry, Freeman Hospital, Newcastle for providing isolates for testing and to Greiner Bio-One, UK for kindly supplying equipment and assay kits.

"STRIVING FOR EXCELLENCE, INSPIRING INNOVATION IN PATIENT CARE WHERE EVERYONE MATTERS"
Department of Microbiology

*Corresponding Author
nathanreading@blueyonder.co.uk
Microbiology Department, City Hospital, Birmingham, UK, B18 7QH

Where EVERYONE Matters