

## Detection of hypermutable strains

Hypermutable or mutator strains are those that have an increased spontaneous mutation rate due to defects in genes involved in DNA repair or error avoidance systems.

Hypermutable strains have been described in natural populations of *E. coli*, *Shigella* spp, *Salmonella* spp. *P. aeruginosa*, *H. influenzae*, *Helicobacter pylori*, *N. meningitidis* and *S. aureus* among others. Hypermutable strains could have an important role in the emergence and spread of antibiotic resistance within bacterial populations, because they are substantially more resistant than nonmutators.

## Detection of *E. coli* hypermutable strains

Galan et al (1) and Conejo et al (2) described a diffusion method for detecting hypermutable strains in *E. coli*. A similar technique may be used with Neo-sensitabs.

MH-Blood agar plates are seeded with 100ul of an overnight Brain Heart Infusion broth culture. Fosfomycin Neo-sensitabs and Rifampicin 30 ug Neo-sensitabs are placed on the surface of the agar plate. Incubation takes place during 24 hours at 35-37 degrees Celsius and the inhibition zones are examined for the presence of colonies inside the inhibition zones.

Interpretation is as follows:

Strong mutators: Strains with > 70 colonies in the Fosfomycin zone and > 10 colonies in the Rifampicin zone.

Weak mutators: Strains with > 30 but <70 colonies in the Fosfomycin zone or > 70 colonies in the Fosfomycin zone but < 10 colonies in the Rifampicin zone.

Nonmutators: Strains with < 30 colonies in the Fosfomycin zone and < 10 colonies in the rifampicin zone.

The same technique should be useful for *Shigella* and *salmonella* strains.

Denamur et al (3) found a high frequency of hypermutable strains in uropathogenic *E. coli* isolates, using nalidixic acid, fosfomycin, spectinomycin and streptomycin. The majority of hypermutable strains yielded colonies inside the inhibition zone (squatted colonies)

Baquero et al (4) found that hypermutable *E. coli* strains were found more frequently in a collection of ESBL-producing isolates, than in non-ESBL *E. coli*.

Ellington et al (5) found that hypermutator phenotypes were found among *E. coli* expressing ESBL CTX-M beta lactamases and they had an increased propensity to fosfomycin resistance.

## Detection of *P. aeruginosa* hypermutable strains

A technique similar to the one described for *E. coli* may be used for *P. aeruginosa* (6,7,8).

MH agar is inoculated with a 0.5 McFarland inoculum or McF 1.0 (mucoïd colonies).

The following Neo-Sensitabs are added to the agar plate: Ceftazidime 30 ug, Ciprofloxacin 5 or 10 ug, Imipenem 10 or 15 ug, Meropenem 10 ug and Tobramycin 10 or 40 ug Neo-sensitabs.

Incubate for 24 hours (36 hours for slow growers) at 35-27 degrees.

The zones of inhibition are examined for the presence of resistant mutants (squatted colonies) inside the zones. When the diameter of the inhibition zones is reduced by > 5 mm (squatted colonies zone compared to the largest zone) the test is positive for hypermutability. With Tobramycin any resistant mutant inside the zone indicates a positive result.

Hypermutable strains will show the presence of resistant mutants inside the zone for 3 or more antibiotics.

Oliver et al (9) found that chronic infections caused by hypermutable *P. aeruginosa* of the sort frequently found in cystic fibrosis patients, will almost invariably be present in a high proportion at the onset of treatment, and consequently these strains should be considered resistant to all agents when they are used as monotherapy.

Henrichfreise et al (10) characterized a multiresistant hypermutable strain of *P. aeruginosa* from an ICU patient using Ciprofloxacin, Tobramycin, Ceftazidime, Imipenem and Meropenem by disk diffusion.

Wayne et al (11) found that the presence of hypermutator *P. aeruginosa* was associated with poorer lung function in cystic fibrosis patients. Mucoïd isolates were more likely to be hypermutators.

Macia et al (12) found that hypermutable *P. aeruginosa* strains from chronic respiratory infections had an increased susceptibility to colistin.

### **Hypermutable *Haemophilus influenzae***

Watson et al (13) found that hypermutable *H. influenzae* with mutations in *mutS* were prevalent in the cystic fibrosis lung environment. Rifampicin, Nalidixic acid and Spectinomycin were used to detect the hypermutable strains. Of 14 hypermutable *H. influenzae* isolates, 12 were from cystic fibrosis sputum.

Perez-Vazquez et al (14) found that 39.3 % of 28 ciprofloxacin resistant *H. influenzae* were hypermutable and conclude that resistance to fluoroquinolones in *H. influenzae* is strongly associated to hypermutability.

### **References**

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