Nükleik Asit Temelli Amplifikasyon Yöntemleri

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TBNET / RESIST-TB 2016

Mevcut testler

Moleküler direnç testleri: Görüşler

Yakın gelecekte MDT

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REVIEW ARTICLE

Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement

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tuberculosis coding for *kat*G, *inh*A, *rpo*B, *emb*B, *rrs*, *rps*L and *gyr*A that are likely related to drug resistance in vivo. Identification of any of these mutations in clinical isolates of *M. tuberculosis* has implications for the management of TB patients, pending the results of in vitro DST. However, false-positive and false-negative results in detecting resistance-associated mutations in drugs for which there is poor or unproven correlation between phenotypic and clinical drug resistance complicate the interpretation. Reports of molecular DST results should therefore include specific information on the mutations identified and provide guidance for clinicians on interpretation and on the choice of the appropriate initial drug regimen.

“MDT raporları saptanan mutasyonun tipi ve buna özel bilgiyi içermeli, bu şekilde klinisyene sonucu yorumlamada ve uygun tedavinin başlatılmasında yardımcı olmalıdır”

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Fenotipik Testler

- Kritik konsantrasyon ??
- İki kritik konsantrasyon : INH, EMB, SM
- MIC belirlenmesi

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	Kritik Kons. (mg/ml)	Serum Kons. (mg/ml)
INH	0.1	7.0
RIF	0.5	10.0
SM	1.0	25-50

Böttger EC, Springer B. Eur J Pediatr (2008) 167:141–148

Isoniazid

- 0.1 mg/l for screening
- Low-level-resistant : R at 0.1 mg/l and S at 1.0 mg/l
- R at 1.0 mg/l and S at 10.0 mg/l need further clinical data for interpretation
- R at 10.0 mg/l : isoniazid is of no clinical use

Springer B, Böttger EC. JCM 2009, Vol47; p. 1773–1780

	RMP	RBT			
rpoB S531L H526mut	–	–	S531L and H526D/Y confer high-level resistance to all rifamycins.[131–136] In contrast, mutation H526L (and possibly H526N/S) only confer low-level resistance to RMP	Strong direct and indirect evidence for association with clinical resistance[128]	More than 95% of RMP-resistant isolates have mutations in the 81-bp core region of the *rpoB* gene. Most studies showed mutations in codons 531 and 526 in 40–65% and 10–40% of RMP-resistant isolates, respectively[127,129,137]
D516mut	–	+	D516mut predominantly affects RMP, but much less so RBT; RBT is still an option for combination chemotherapy[132–134,136,138]		Most studies showed codon 516 and 533 mutations in 5–32% and 2–5% of RMP-resistant isolates, respectively.[127,129,137] Their frequencies are probably underestimated, as low-level resistant isolates may be tested as phenotypically susceptible[67]
L533mut	+	+	L533mut affects susceptibility to all rifamycin only slightly; RMP and RBT are still an option for combination chemotherapy[134,139–141]		

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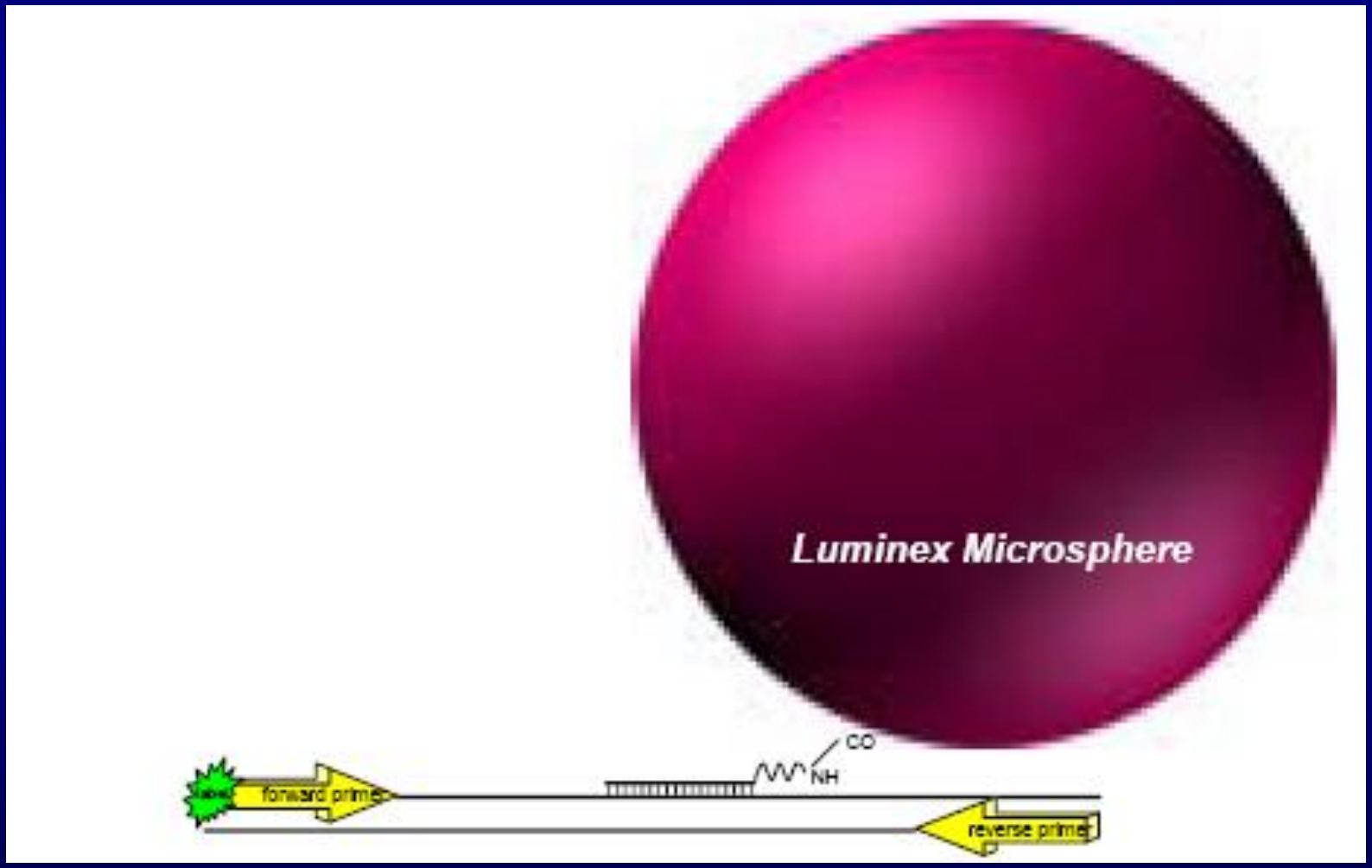
- S531L ve H526 D/Y mut : Yüksek düzey direnç kodlar, ama H526L/N/S mut: düşük RIF direnci
- D516mut : RIF düşük direnç, RBT'e ise etki etmeyebilir

İstanbulda En Sık Rastlanan Mutasyonlar (20)

505 TTC / TGC (1)	F505C	
509 AGC / ACC (2)	S509T	(-)
510 CAG / CTG (1)	Q510L	
511 CTG / CCG (2)	L511P	
513 CAA /CTA (1)	Q513L	
513 CAA / AAA (1)	Q513K	
516 GAC / TAC (2)	D516Y	
516 GAC / GTC (4)	D516V	3.5%
del519 AAC/--- (1)		
526 CAC / TAC (4)	H526Y	3.5%
526 CAC / CTC (1)	H526L	
526 CAC / CGC (2)	H526R	
526 CAC / GAC (3)	H526D	2.6%
526 CAC / TCC (1)	H526F	
530 CTG / ATG (1)	L530M	(-)
531 TCG / TTG (82)	S531L	71.3%
531 TCG / TGG (2)	S531W	
531 TCG / TTC	S531F	
533 CTG / CCG	L533P	(-)
561 ATC / GTC		(-)

Mikrokürecik Bazlı Hibridizasyon Testi



	EMB			
*emb*B M306mut	+	M306mut mostly confers low- to moderate-levels of drug resistance, the clinical implications of which are not clear[69,70,150–154]	There have been no studies of the direct effect of *emb*B306 mutations on clinical resistance	In various studies, 20–88% of EMB-resistant isolates had *emb*B306 mutation[108,127,155–159]

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Mutation	Drug*		Association with in vitro phenotypic resistance	Association with clinical resistance	Frequency among strains categorised as resistant on the basis of critical concentration testing‡
	INH	ETH			
katG S315T	–	+	S315T confers high-level INH resistance (MIC >1 mg/l), but does not affect susceptibility to ETH[69,70,119–121] Note: there are additional mutations in *inh*A or *eth*A, which confer ETH resistance[122]	Indirect evidence strongly suggests that high-level resistance affects clinical outcomes. *kat*G S315T mutations are associated with multidrug resistance (see e.g.[119]). Limited data on direct association between *kat*G S315T mutation and clinical outcome suggest increased risk of first-line treatment failure, death and relapse[123,124]	In a systematic review of 52 studies, 5–98% of INH-resistant isolates showed *kat*G S315T mutations (median 64%, interquartile range 54–79) (Hooijer et al. unpublished)

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*inh*A −16G −15T −8A/C	+	−	*inh*A promoter mutations confer low-level INH resistance (MIC <1 mg/l), but significantly affect ETH susceptibility[69,70,119–121,123] Note: there are additional mutations in the structural *inh*A gene, which together with *inh*A promoter mutations result in INH MIC levels >1 mg/l[125]	Limited direct and indirect data, suggesting no effect on cure rates for standard first-line treatment.[123,124] One study showed increased relapse rates with INH-EMB (6 months) in the continuation phase;[138] *inh*A promoter mutations are not associated with multidrug resistance when compared to the *katG* S315T mutation,[119] but have been associated with XDR-TB in South Africa[126]	In various studies, 12–42% of INH-resistant isolates had *inh*A promoter region mutations[113,119,127–130]

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Mevcut testler

- Genotype MDRTB*plus* ve *sl*
- Xpert
- Anyplex II Seegene
- Nipro

Moleküler RIF Direnç Testi

Sens %95 Spes: %98 n=12000 RIF Direnci : %3

Pos Pred Değer= % 59.5

Dirençli moleküler sonuçlar fenotipik İDT ile onaylanmalı

- **MDR-TB tedavi rejimlerinde başarı : %60**
- **%40: tedavi başarısızlığı, terk, ölüm**

van Deun A et al. 2010 Am J Respir Crit Care Med; 182: 684–692.

Daha fazla MDT = Daha fazla MDR tanısı

= Daha fazla MDR tedavisi

= Daha fazla başarısız MDR tedavisi

= Daha fazla XDR

Salgın Bileşkeleri

- Aktif hastanın bulaştırıcılık süresi
- Bulaştırıcı kişinin temas ettiği yatkın kişi sayısı
- Enfekte kişinin bulaştırıcı hale dönüşmesi olasılığı

Tanı gecikmesi

Tanı gecikmesi = Hasta gecikmesi + doktor gecikmesi

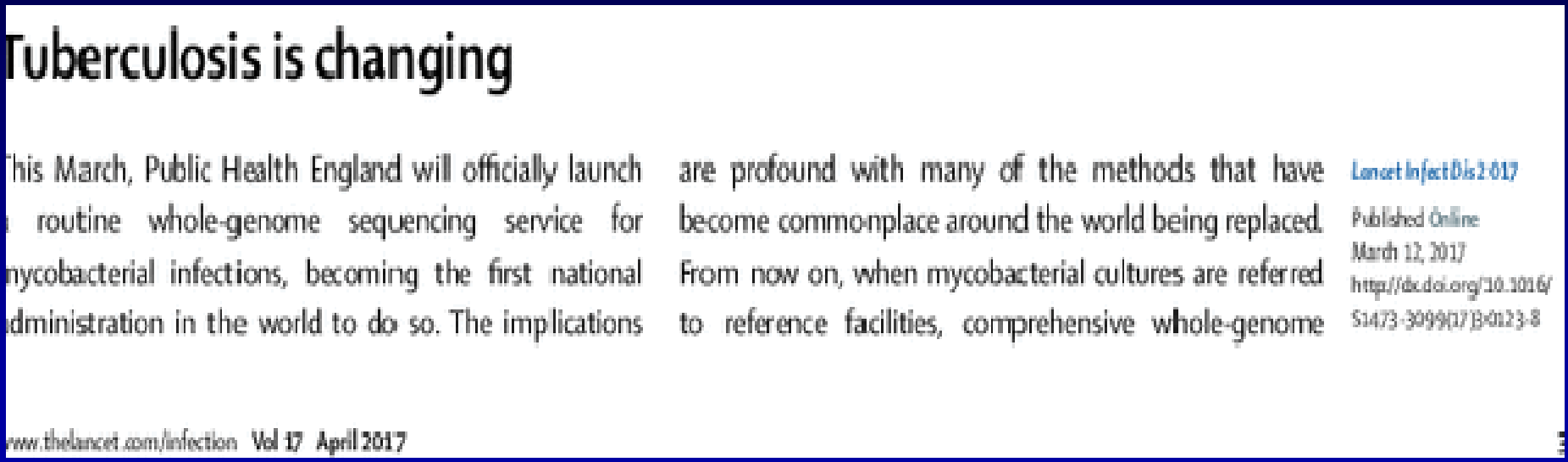
Moleküler epidemiyoloji bazlı ve aktif olgu yakalama DOTS stratejisine eklenmelidir

Türkiye'de

- n=10000 TB hastası 7000 PTB
- %50 Yayma (+)= **3500** moleküler İDT
- 7000 hasta yakalamak için **100000-150000** mol İDT

Yakın Gelecekte MDT

- Yeni nesil WGS yöntemleri



Tuberculosis is changing

This March, Public Health England will officially launch a routine whole-genome sequencing service for mycobacterial infections, becoming the first national administration in the world to do so. The implications are profound with many of the methods that have become commonplace around the world being replaced. From now on, when mycobacterial cultures are referred to reference facilities, comprehensive whole-genome

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…….comprehensive whole-genome sequencing-based reports will be returned within 5–7 days. Reports will detail mycobacterial species, and predict drug susceptibility and genetic relatedness for the *Mycobacterium tuberculosis* complex.

Walker TM et al. Lancet Infect Dis April 2017


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