Determination of recurrent and polyclonal infections in melioidosis

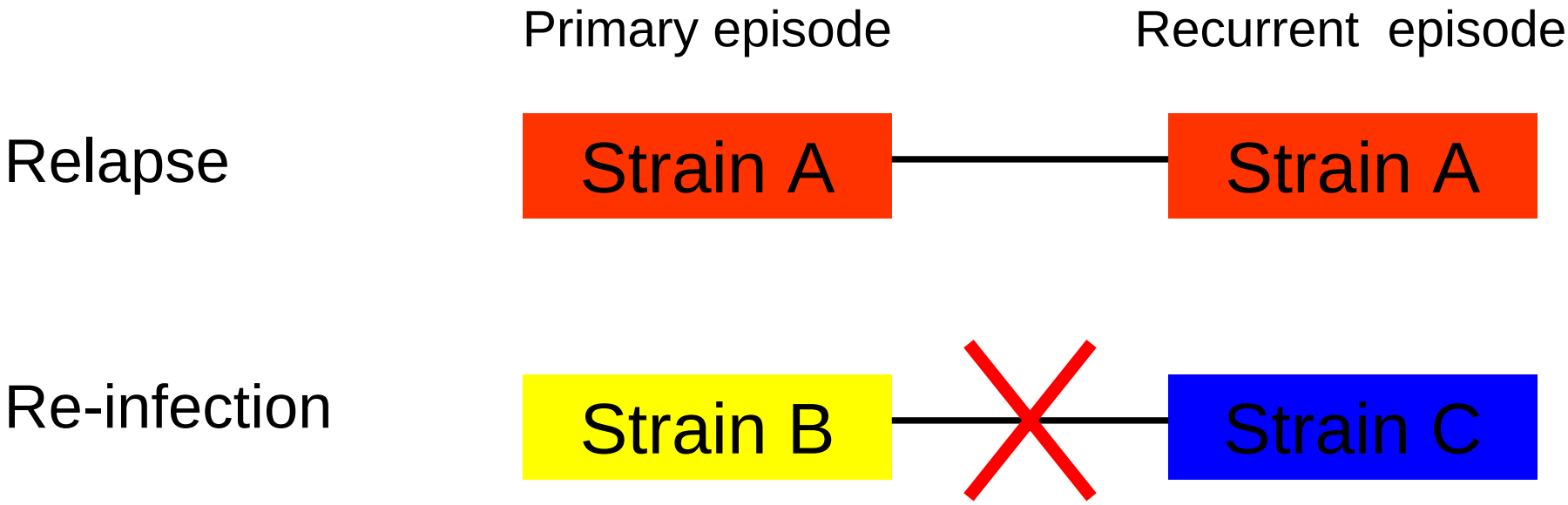
Direk Limmathurotsakul

Mahidol-Oxford Tropical Medicine Research Unit,
Mahidol University, Thailand

Recurrent melioidosis

- Recurrent melioidosis following primary disease is common (10-25%)
- Death occurs in 30% of cases
- Previous small studies have shown recurrence to be associated with treatment duration, antimicrobial drug used and disease severity
- Previous studies analyzed all cases of recurrence as relapse

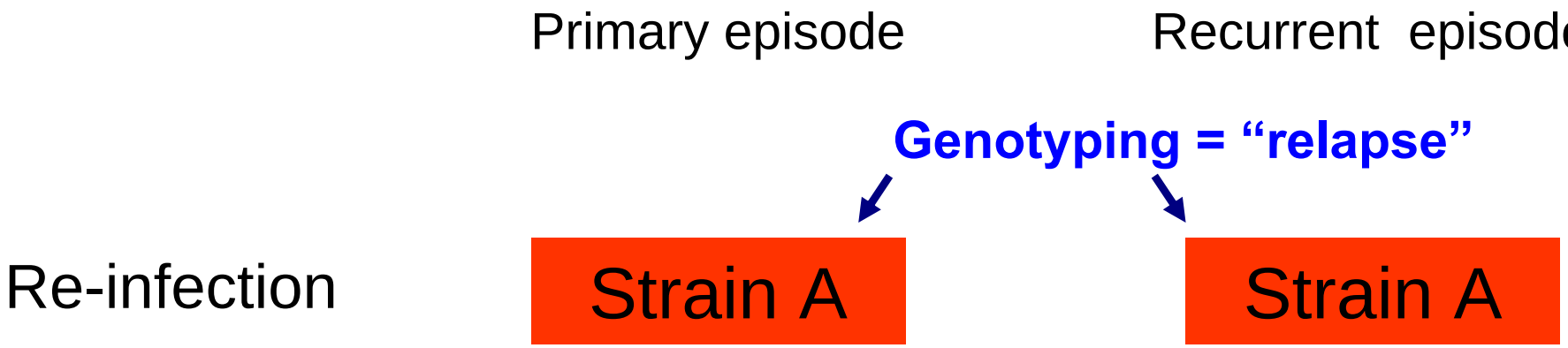
Relapse versus re-infection



A large study in Thailand indicated that around 25% of recurrent melioidosis was due to re-infection*

*Maharjan B, Chantratita N, Vesaratchavest M et al. *Recurrent melioidosis in patients in northeast Thailand is frequently due to reinfection rather than relapse.* J Clin Microbiol 2005; 43:6032-4.

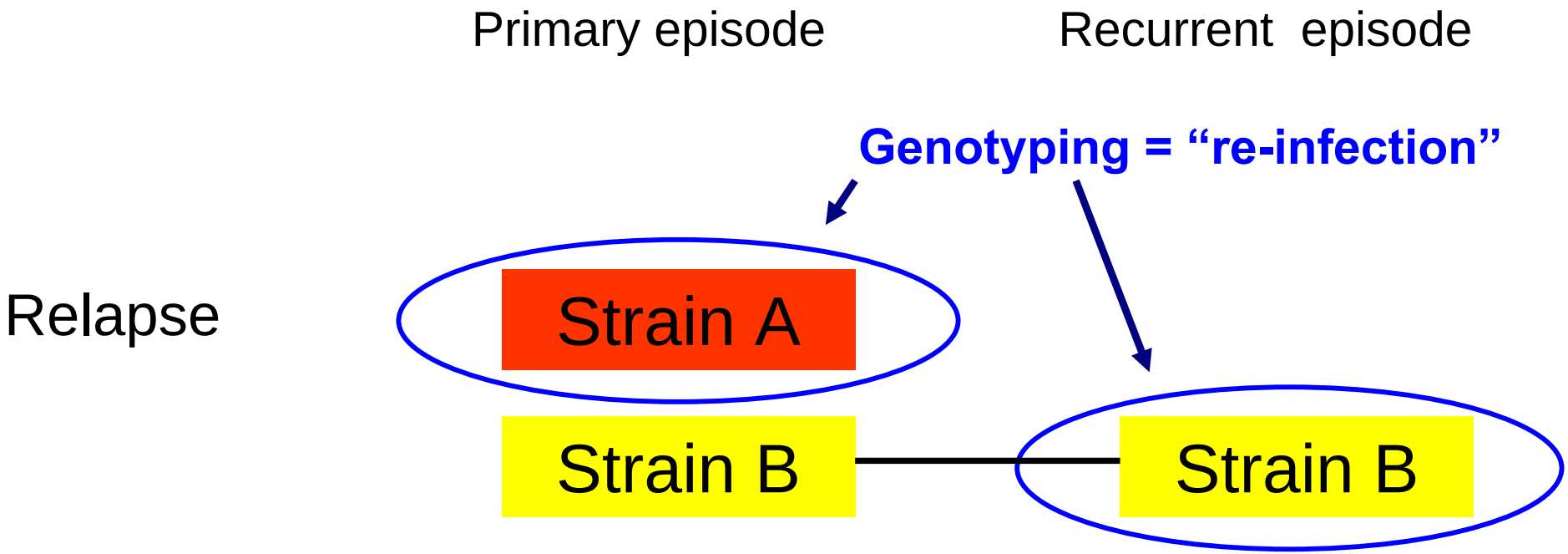
Chance of re-infection with same genotype



- In the MLST database (www.mlst.net), 236 STs have been assigned to 554 *B. pseudomallei* Thai isolates, of which 148 (63%) are represented by a single isolate
- A recent environmental study has demonstrated very high genetic diversity (talk by Vanaporn Wuthiekanun and *)

* Wuthiekanun V, Chantratita N, Limmathurotsakul D, et al. *Genetic diversity and microevolution of B. pseudomallei in the environment.* PLoS Negl Trop Dis , under review

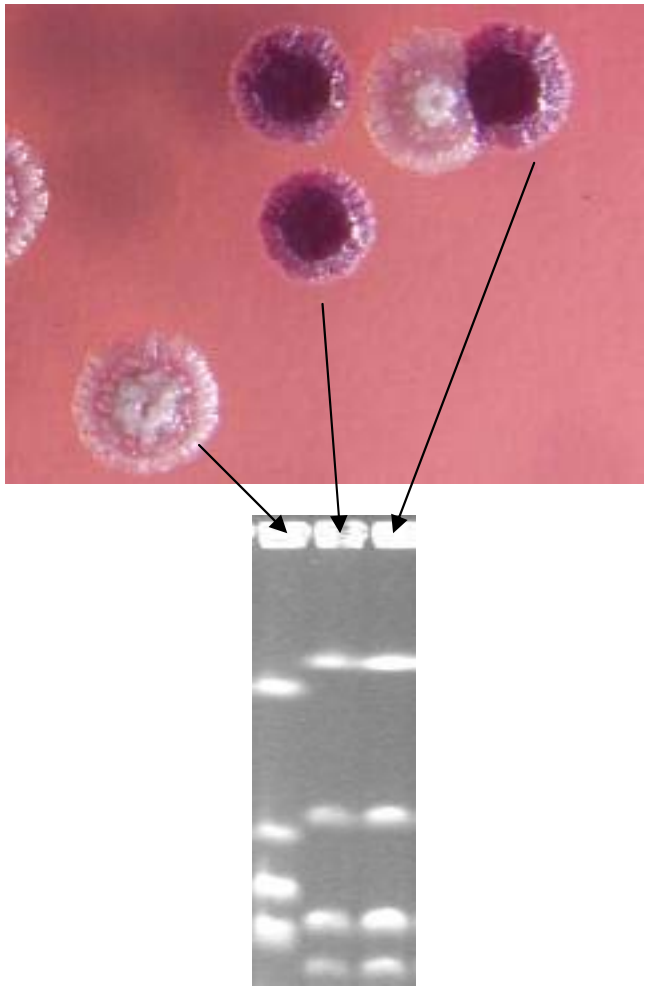
Chance of relapse with different genotype



- Confounding factor : rate of simultaneous infection with more than one strain of *B. pseudomallei* on primary episode

Using genotyping to determine simultaneous infection with different strains

PFGE



MLST

*ace*1 :
CCGCAGTCTTT
CTCAGGAGGAT
CCGTCAGCCGG
GCCGTCC….

*glt*B1 :
TCATGGCCG
AGGCTCCAC
CTATTCGGG
CCTGT….

ST 1	1-1-1-2-4-2-1
ST 10	1-1-13-1-1-1-1

Polyclonal infection

Material and methods

- 10 colonies from each culture positive specimen from each patient were collected. A combination of PFGE and MLST were used to determine polyclonal infection

Results

- **133 patients** were enrolled prospectively
- 2,058 colonies (215 samples) were examined by PFGE
- **Only 2 of 133 patients were infected with two different strains of *B. pseudomallei*** (1.5%, 95% CI 0.2 to 5.3%)

* Limmathurotsakul D, Wuthiekanun V, Chantratita N et al. *Simultaneous infection with more than one strain of B. pseudomallei is uncommon in human melioidosis.* J Clin Microbiol 2007; 45: 3830-3832.

Objectives

- Define specific risk factors associated with relapse and re-infection
- Compare clinical manifestations between relapse and re-infection
- Evaluate whether clinical manifestations of initial episode are the same in relapse and re-infection
- Develop simple scoring system to differentiate re-infection from relapse based on basic clinical information

Materials and Methods

Inclusion criteria

- Age > 14 yrs
- culture-confirmed melioidosis
- survived to receive oral treatment

Time: 1986 to 2005 and follow-up to 2007

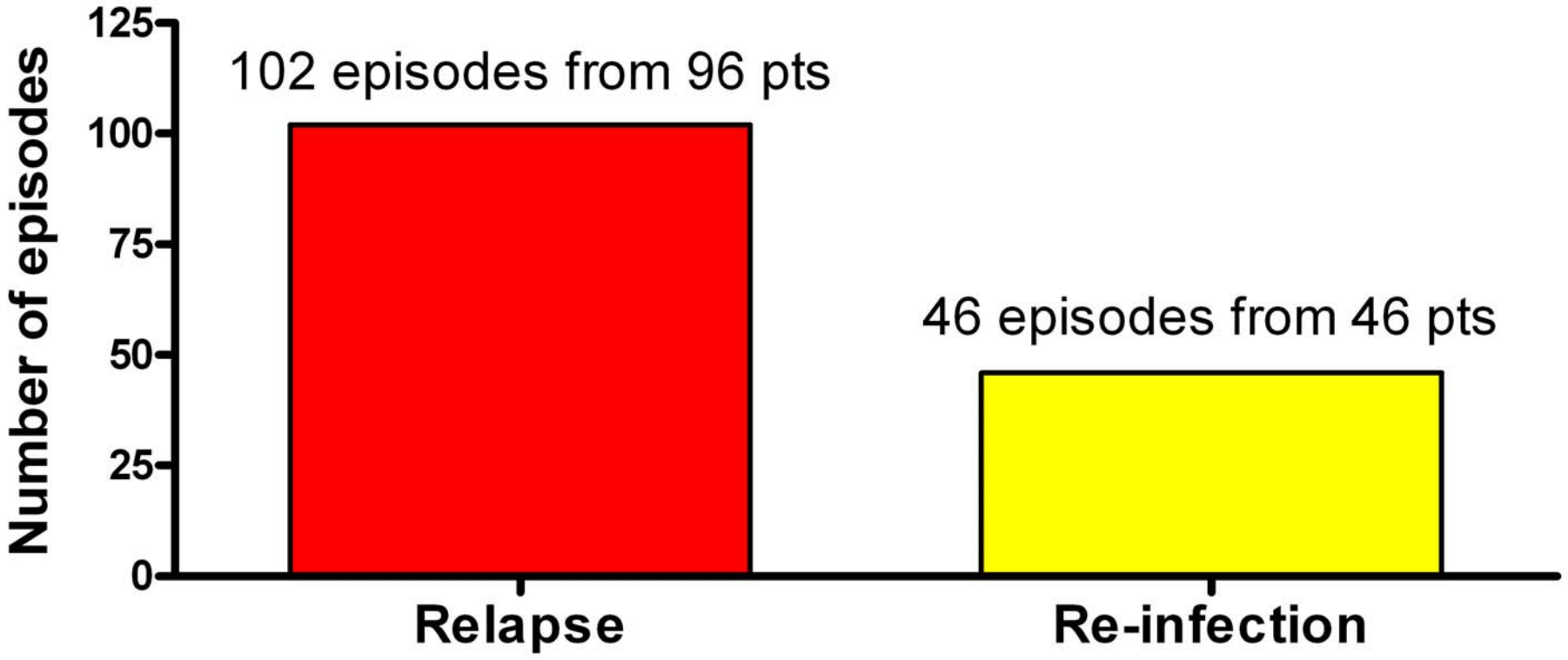
Location: Sappasithiprasong Hosp., Thailand

Genotyping

- Isolates obtained from the first and recurrent episodes were compared using a combination of PFGE and MLST

Outcome

Recurrent melioidosis occurred in 141 of 1,001 adult patients who survived the primary infection



Specific risk factors

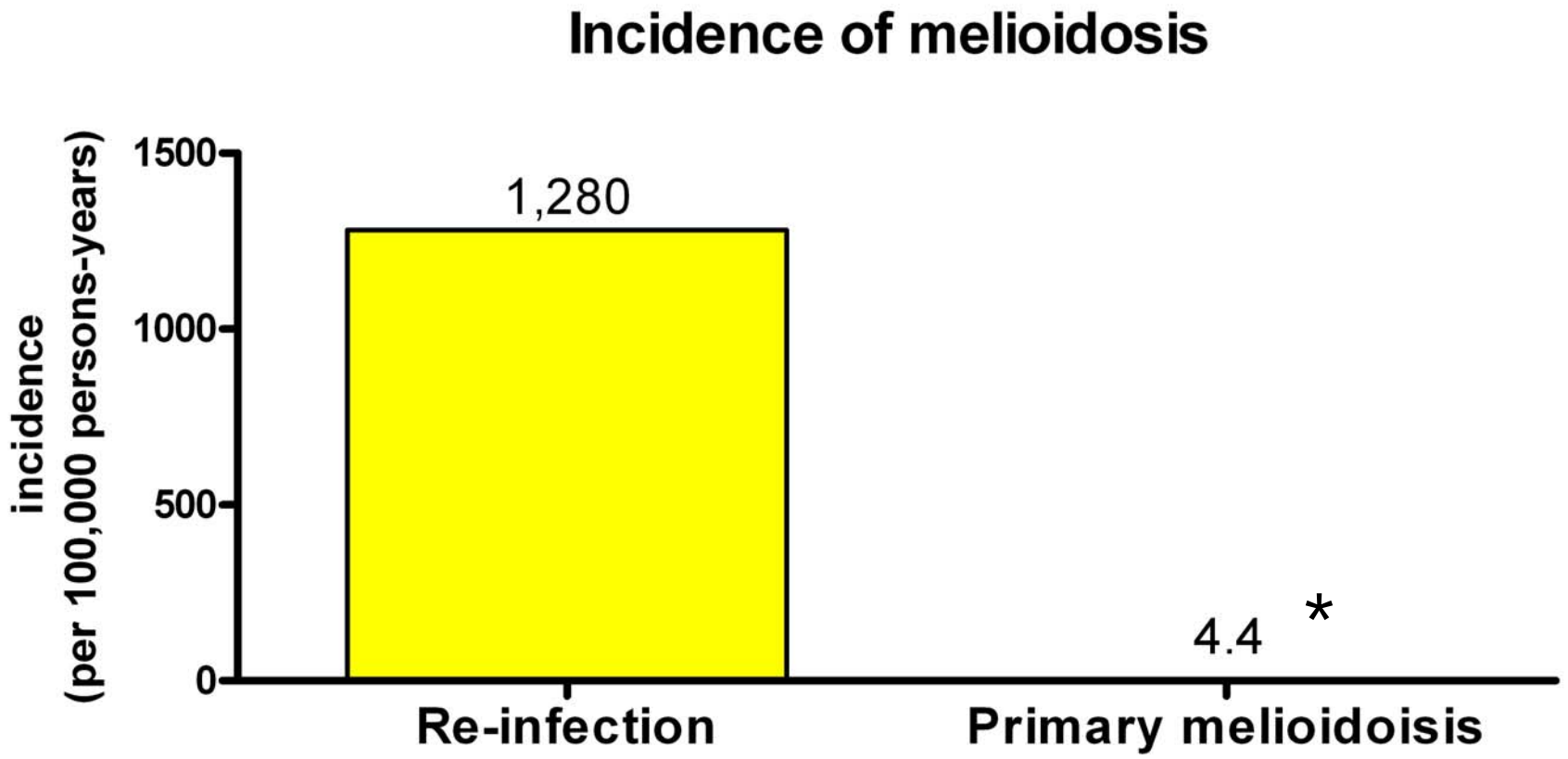
Risk factors for relapse

Prognostic factors	HR (95%CI)	*P* value
Distribution : multifocal	2.05 (1.09-3.87)	.03
Blood culture positive	1.84 (1.17-2.89)	.01
First oral treatment regimen		
: 4-drug and 3-drug regimens	1.0	
: Amoxycillin-clavulanic acid	2.09 (1.14-3.83)	.02
: Other regimens	3.16 (1.63-6.15)	.001
Duration of treatment (months)	0.87 (0.75-1.01)	.06
Relapse during treatment		
: Standard oral regimen	1.0	
: Other regimens	2.31 (1.11-4.82)	.03

Limmathurotsakul D, Chaowagul W, Chierakul W et al. *Risk factors for recurrent melioidosis in northeast Thailand.* Clin Infect Dis 2006; 43:979-86.

Risk factors for re-infection

No specific risk factors were found



* Suputtamongkol Y, Hall AJ, Dance DA, et al.
The epidemiology of melioidosis in Ubon Ratchathani, norteast Thailand.. Int J Epidemiol 1994; 23:1082-90.

Relapse vs Re-infection

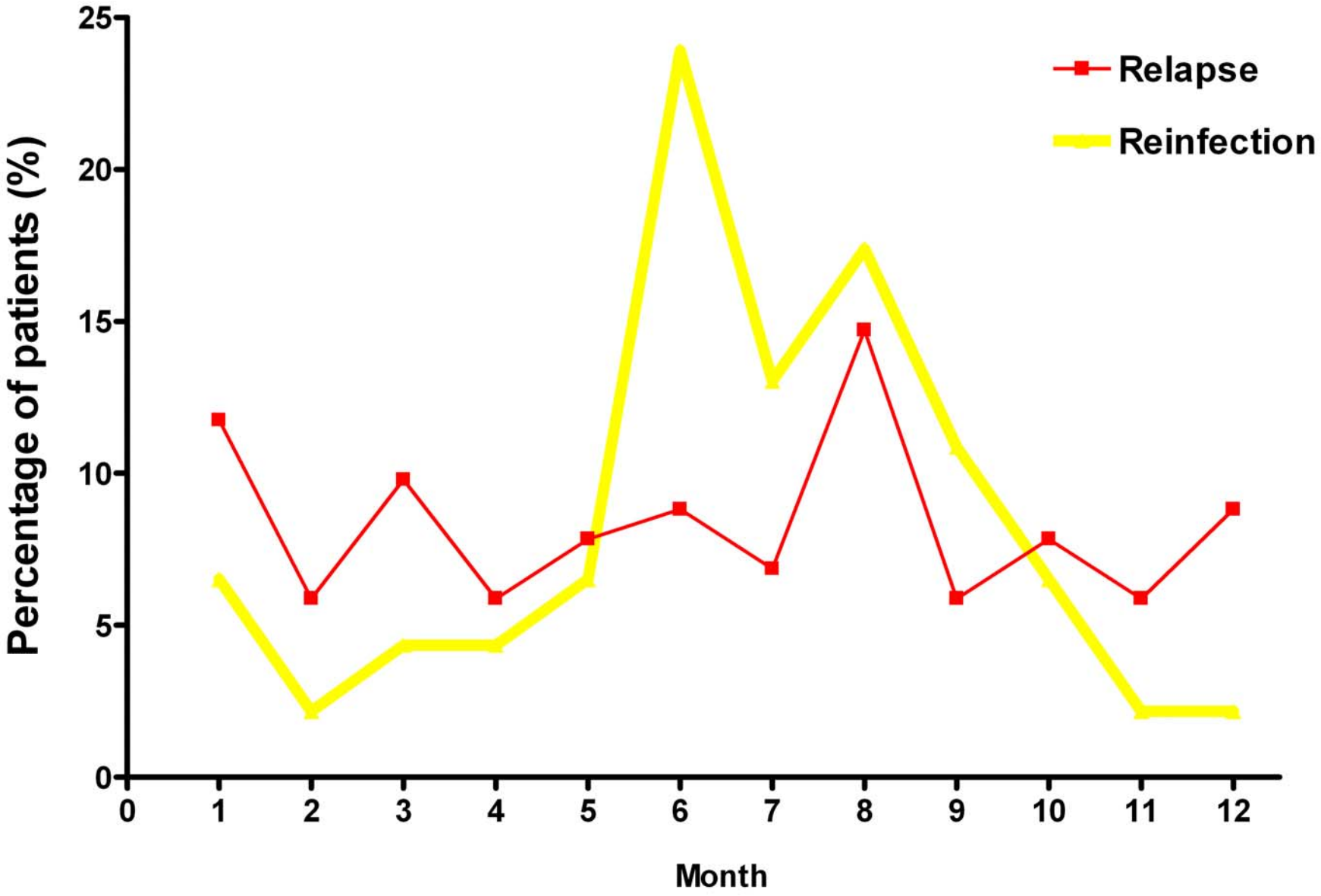
Relapse versus re-infection (1)

Variable	Relapse (N=96)	Re-infection (N=45)	*P* value
Time to recurrence (months)	6 (2-17)	27 (13-46)	<0.001
Presentation in rainy season (June to November)	50%	73%	.01
Acute impairment of renal function (GFR on admission)	58 (34-93)	40 (20-60)	.01
Bacteremia	47%	58%	.23
Pneumonia	31%	31%	.99
Liver abscess	19%	18%	.89
Splenic abscess	16%	13%	.72
Skin or soft tissue infection	32%	36%	.70

Relapse versus re-infection (2)

Variable	Relapse (N=96)	Reinfection (N=45)	*P* value
Antimicrobial resistance:			
Ceftazidime	0	0	-
Amoxycillin-clavulanic acid	2%	0	.33
Carbapenem	0	0	-
Doxycycline	2%	0	.33
TMP-SMX	14%	13%	.97
Treated with ceftazidime	70%	93%	.002
Treated with carbapenem	11%	11%	.95
Died	19%	27%	.28

Month of presentation for relapse and re-infection



Repeated presentation

Clinical features of relapse versus primary episode (N=96 patients)

Variable	Primary	Relapse	(%repeat + %new)	*P* value (Kappa)
Blood culture +ve	53%	47%	(30% + 17%)	.02
Pneumonia	39%	31%	(20% + 11%)	<.001
Liver abscess	30%	19%	(13% + 6%)	<.001
Splenic abscess	34%	16%	(11% + 5%)	.002
Skin or soft tissue infection	29%	32%	(16% + 16%)	.002
Arthritis	13%	14%	(2% + 12%)	.37
Osteomyelitis	3%	7%	(1% + 6%)	.16

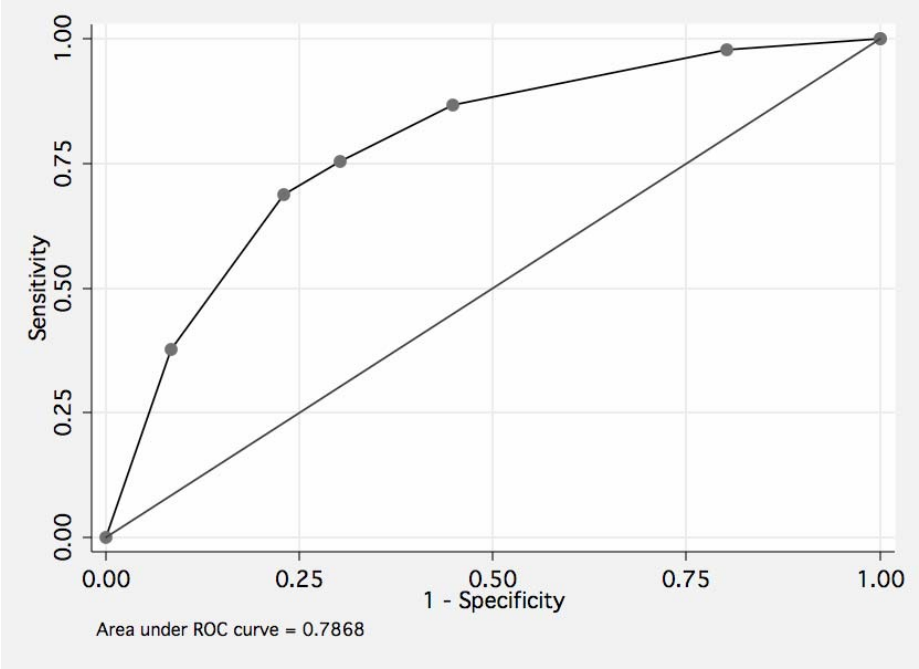
Ubon patients, 1106 and 1710

Patient	1106a	1106b	1710a	1710b
Time of presentation	Jul 93	Aug 96	Sep 96	May 99
Blood culture +ve	-ve	-ve	**+ve**	**+ve**
Pneumonia	No	No	Yes	No
Liver abscess	**Yes**	**Yes**	No	No
Splenic abscess	No	No	No	No
Skin or soft tissue infection	No	No	Yes	No
Arthritis	No	Yes	No	No
Osteomyelitis	No	No	No	No

Clinical features of re-infection versus primary episode (N=45 patients)

Variable	Primary	Re-infection (%repeat + %new)	*P* value (Kappa)
Blood culture +ve	44%	58% (29% + 29%)	.19
Pneumonia	47%	31% (22% + 9%)	.01
Liver abscess	22%	18% (11% + 7%)	.001
Splenic abscess	24%	13% (9% + 4%)	.005
Skin or soft tissue infection	18%	36% (9% + 27%)	.17
Arthritis	11%	18% (2% + 16%)	.45
Osteomyelitis	0%	2%	-

Re-infection score



AOC = 0.79 ± 0.04

Re-infection score = sum of

3 points for time to recurrence > 1 yr

1 point for presentation in rainy season

1 point for acute impairment of renal function

(GFR<60 mL/min per 1.73 m^2)

Cut-off score	≥3	≥4	≥5
Sensitivity	76%	69%	38%
Specificity	70%	77%	92%

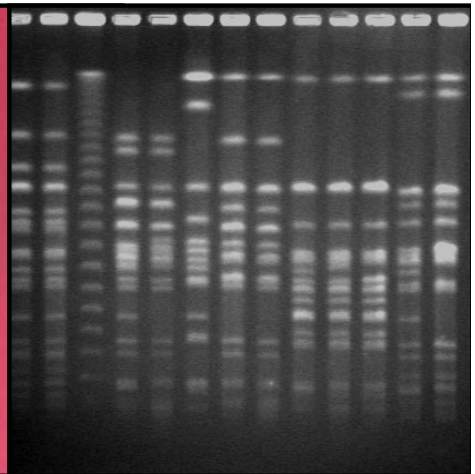
Conclusions

Factors	Relapse	Re-infection
Risk factors	eradicative therapy bacteremia multifocal infection	None
Timing	< 1st year, not seasonal	> 2nd year, rainy season
Presentation	Site of infection often the same as primary episode	Site of infection often the same as primary episode Acute renal impairment
Antimicrobial resistance	4%	None
Recommended treatment	Standard regimens	Standard regimens

Recommendations

- Patients with primary disease have a much higher risk of re-infection, and prevention is necessary
- Re-infection is not uncommon, and genotyping is required to differentiate between relapse and re-infection



Acknowledgements

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Previous Wellcome unit colleagues

Wellcome Trust

THE END

Statistical Analysis

Define specific risk factors associated with relapse and re-infection	Multivariate Cox proportional hazards regression analysis for relapse free-survival Repeat analysis with re-infection as an outcome
Compare clinical manifestation	**Chi-square, Fisher's test, and Mann-Whitney test**
Evaluate the homogeneity of clinical manifestation	McNemar and Kappa index
Create simple tool to identify re-infection	**Multivariate logistic regression analysis and construct a scoring system**

Variables in the analysis

- Demographic (age, sex, occupation)
- Underlying disease (diabetes, renal impairment, thalassemia, steroid abuse)
- Disease distribution (organ involvement, microbiological culture result)
- Treatment (initial parenteral and oral maintenance treatment)
- Duration of treatment (time-dependent variable)
- Time of recurrence (stratified variable)

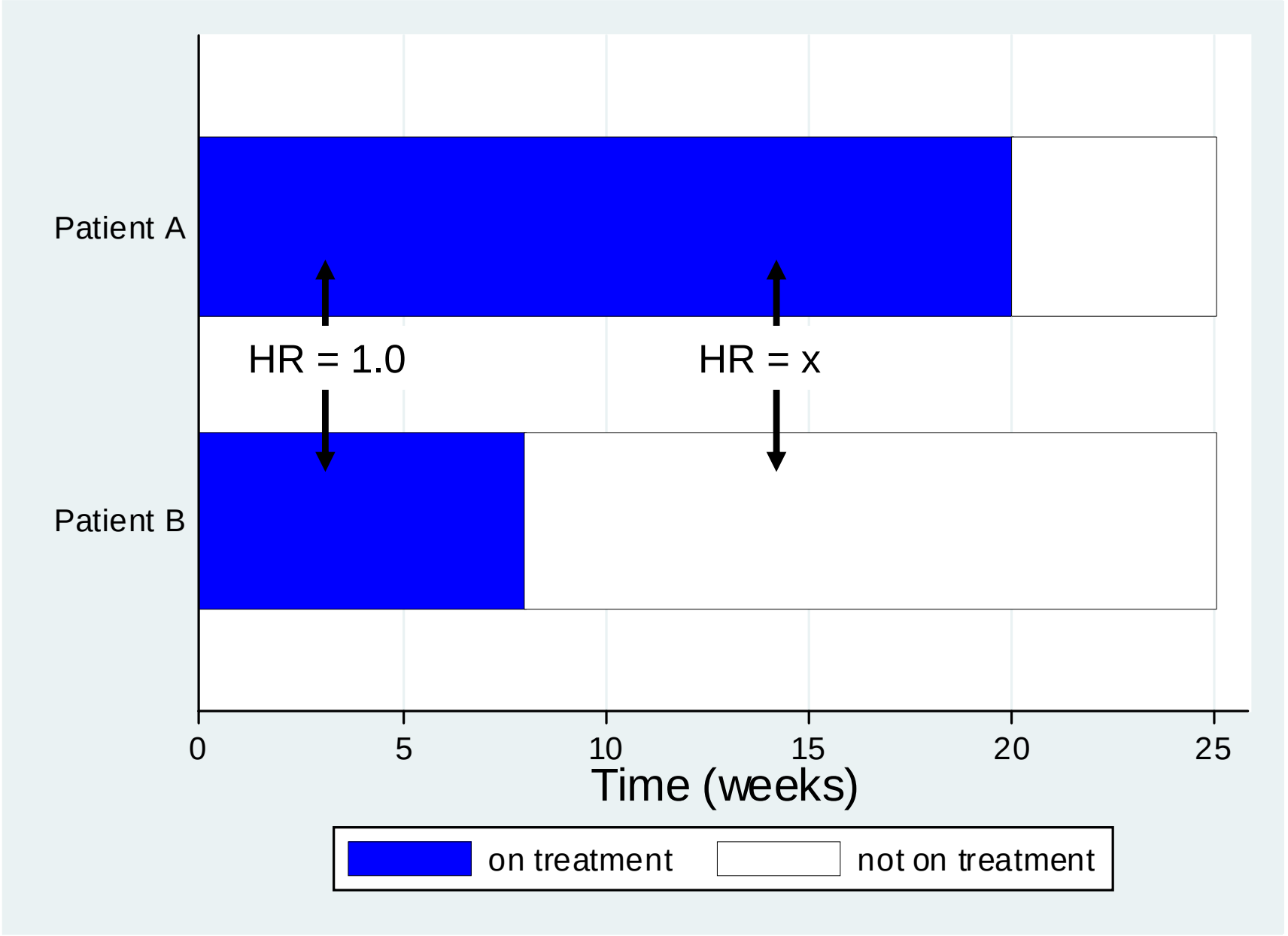
Recurrent event model

Model	Time interval	Failure	Stratum
Conditional A	(0,9]	1	1
	(9,13]	1	2
Aim: full time course	(13,28]	1	3
	(28,31]	0	4
Conditional B √	(0,9]	1	1
(resets the clock)	(0,4]	1	2
Aim: gap time between events	(0,15]	1	3
	(0,3]	0	4

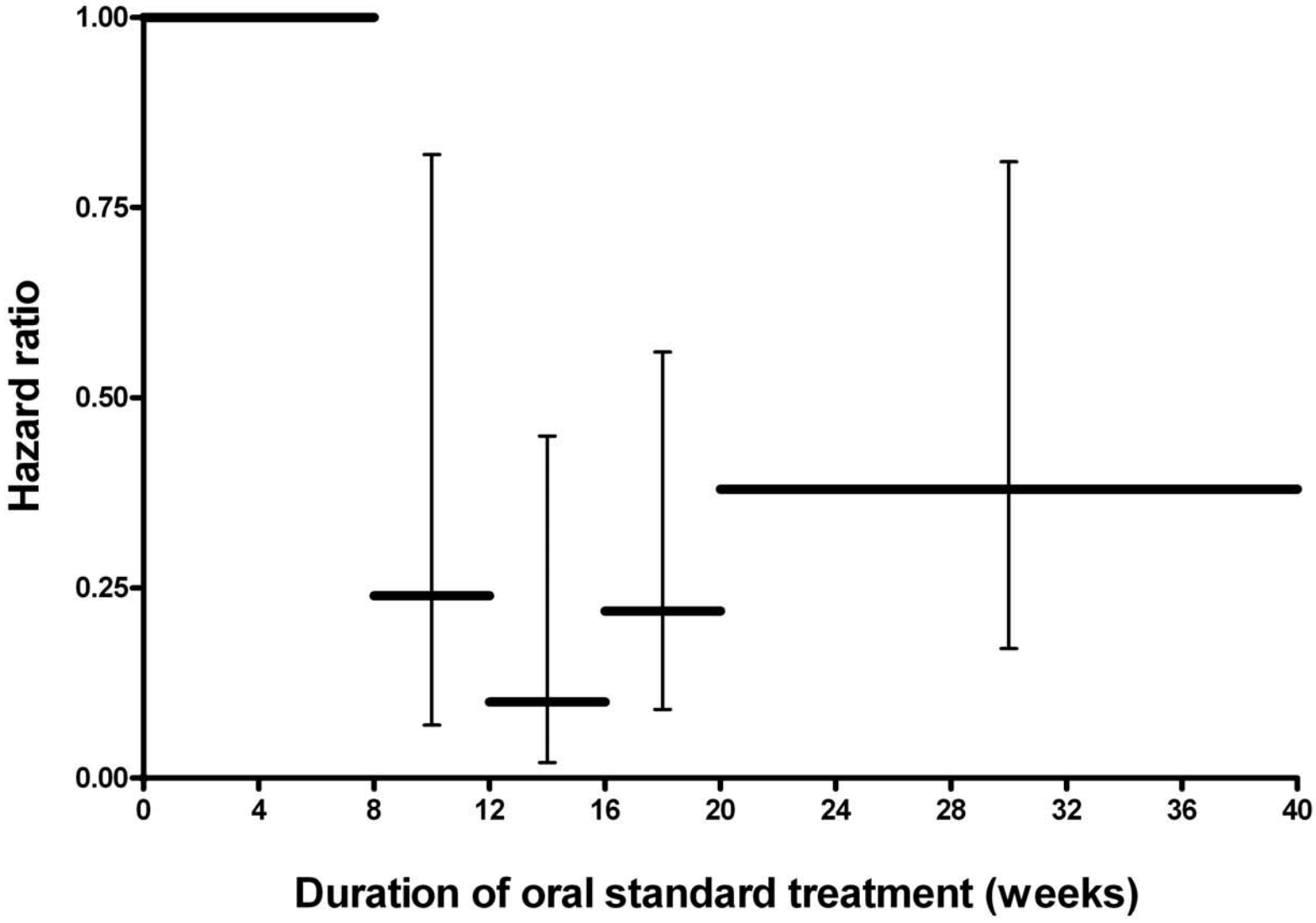
Time to recurrence

- Time start: complete satisfactory parenteral treatment and start oral eradicative treatment
- Time stop:
  - Clinical onset of recurrent melioidosis (failure or censored)
  - Died from other causes (censored)
  - Last follow-up with survival outcome (censored)
- Parenteral treatment during clinical recurrence was treated as gap.

Treatment as time-varying covariate



Estimated of HR and 95% CI for duration of standard oral antimicrobial treatment



McNemar & Kappa

Bacteremia	Relapse +ve	Relapse -ve
Primary +ve	a	b
Primary -ve	c	d

- McNemar = $\frac{(b - c)^2}{b + c}$

- Kappa = $\frac{(a+d) - q}{N - q}$

- q = number of cases expected in "a" and "d" by chance

$$q = \frac{(a+b)*(a+c)}{N} + \frac{(d+b) * (d+c)}{N}$$