Transparency Declaration

The authors declare the absence of dual or conflicting interests.

References

1. Hsieh SY, Tseng CL, Lee YS *et al.* Highly efficient classification and identification of human pathogenic bacteria by maldi-tof ms. *Mol Cell Proteomics* 2008; 7: 448–456.
2. Seng P, Drancourt M, Gouriet F *et al.* Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009; 49: 543–551.
3. Maier T, Schwarz G, Kostrzewa M *et al.* Rapid identification of bacteria from blood cultures using MALDI-TOF MS. *48th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC: American Society for Microbiology, 2008; Abstract D-302.
4. Stevenson LG, Drake SK, Murray PR. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2009; 48: 444–447.
5. La Scola B, Raoult D. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. *PLoS ONE* 2009; 4: E8041.

Does RecA have a role in *Borrelia recurrentis*?

S. J. Cutler, I. J. Rinky and E. M. Bonilla

School of Health & Bioscience, University of East London, London, UK

Abstract

Genomic sequencing of two relapsing fever spirochaetes showed truncation of *recA* in *Borrelia recurrentis*, but not in *Borrelia duttonii*. RecA has an important role among bacteria; we investigated whether this characteristic was representative of *B. recurrentis*, or an artefact following *in vitro* cultivation. We sequenced *recA* directly from samples of patient with louse-borne relapsing fever (*B. recurrentis*) or tick-borne relapsing fever (*B. duttonii*). We confirmed the premature stop codon in seven louse-borne relapsing fever samples, and its absence from three tick-borne relapsing fever samples. Furthermore, specific signature polymorphisms were found that could differentiate between these highly similar spirochaetes.

Keywords: *Borrelia duttonii*, *Borrelia recurrentis*, louse-borne relapsing fever, RecA, relapsing fever, tick-borne relapsing fever

Original Submission: 31 December 2009; **Revised Submission:** 29 March 2010; **Accepted:** 19 April 2010

Editor: M. Drancourt

Article published online: 28 April 2010

Clin Microbiol Infect 2011; **17:** 195–197

10.1111/j.1469-0691.2010.03249.x

Corresponding author: S. J. Cutler, School of Health & Bioscience, University of East London, Water Lane, Stratford, London, E15 4LZ, UK

E-mail: s.cutler@uel.ac.uk

Analysis of the full genomic sequences of two relapsing fever spirochaetes that remain prevalent in East Africa showed that these organisms were highly similar [1]. Indeed, it would appear that the louse-borne *Borrelia recurrentis* was derived from the tick-borne *Borrelia duttonii*, or that both evolved from a similar common ancestral strain. Furthermore, the genome of *B. recurrentis* appeared to be a degraded subset of that of *B. duttonii*, containing many pseudogenes that have with functional counterparts in *B. duttonii* [1]. One such polymorphism is the apparent truncation of *recA* seen in *B. recurrentis*; this gene remains functional in *B. duttonii*. As there are implications regarding the efficacy of DNA repair and recombination without functional RecA, we explored whether this feature was unique to the cultivated strain A1 from which the genomic sequencing was performed, or whether this was a characteristic of *B. recurrentis* in general.

Given that greater diversity has been seen among *B. duttonii* detected in either ticks or in clinical samples than in cultivable isolates [2], we decided to characterize *recA* genes directly from patient samples rather than looking only at successfully cultivated isolates. Our clinical samples were obtained from patients diagnosed with either louse-borne relapsing fever from Ethiopia (n = 30) or from those with tick-borne relapsing fever from Tanzania (n = 4). Diagnosis was confirmed by demonstration of the spirochaetes by examination of Giemsa-stained or Field's-stained blood films, and subsequently confirmed by real-time PCR with primers (Table 1) against the flagellin gene. These spirochaetes were further typed by sequence analysis of their 16S RNA–23S RNA intragenic spacer region (Table 1) [2,3]. Primers designed against the published *recA* gene sequence were used to characterize the sequence of this gene directly from clinical samples. Sequencing was performed in both directions, using BigDye 3.1 chemistry on an ABI3700 at the Queen Mary's University of London genomic sequencing centre, London.

TABLE 1. Primers used for detection and sequencing during this study

Primer/Probe name	Specificity	Sequence, 5′–3′
FlaF	Flagellin forward	CTAGTGGGCATAGAATTAATCGTGC
FlaR	Flagellin reverse	GCTTGGGATAACCCTCTAATTTGA
FlaP	Flagellin probe	Fam-TGGTATGGGTGTTGCTGGGAAAATTACG-bhq1
RF-RecA-F	recA forward	TCGTCTTGAAGTTAGAAAGGTTGAGCA
RF-RecA-R	recA reverse	GTTCATTTGCAAGGGCTTTTTCATT
CompF	recA forward	CCTCACTCTTCGAAATATCAACTG
CompR	recA reverse	GGAATTGCGGCTTTTATTGA
RecA nest	recA forward	TACGAAAAGGAGGTGCCACT
RecA nest	recA reverse	AAGAAAAAGCCATTGAACTTG
RF-RecA-F aug1	recA forward	CTTTGATGCCACAAGCAGAA
RF-RecA-R aug1	recA reverse	CCTCACTCTTCGAAATATCAACTG
RF-RecA-F aug2	recA forward	AGAAATTGATGGCGAGATGG
RF-RecA-R aug2	recA reverse	TTTTCCTCACTCTTCGAAATATCA
RF-RecA-F aug3	recA forward	CTTTGATGCCACAAGCAGAA

TABLE 2. Polymorphisms between *recA* gene sequences of *Borrelia recurrentis* and *Borrelia duttonii*

SNP location	*B. recurrentis*	Amino acid	No. of samples	*B. duttonii*	Amino acid	No. of samples
47	TTA	Leu	0[a]	TTG	Leu	0[a]
169	TTT	Phe	17	TCT	Ser	0[a]
395	CCC	Pro	26	CCT	Pro	4
413	GCT	Ala	12[a]	GCC	Ala	4
	GCC	Ala	17			
487	ATG	Met	30	ACG	Thr	4
578	GCT	Ala	30	GCC	Ala	4
597	GCT	Ala	30	ACT	Thr	4
839	GGC	Gly	7	GGT	Gly	3
860	GCT	Ala	7	GCG	Ala	3
913	TAG	Stop	7	TGG	Trp	3
977	AAT	Asn	7	AAG	Lys	3

SNP, single-nucleotide polymorphism.
[a]SNP located from sequenced cultivated strains only [1].
B. duttonii recA sequences have been deposited under GenBank numbers GU371222–GU371225, and those for *B. recurrentis* under numbers GU371226–GU371255.

We successfully obtained partial *recA* sequence data from 30 samples of *B. recurrentis* and four of *B. duttonii*. These data were variable in length, with only seven *B. recurrentis* and three *B. duttonii* samples covering the region containing the truncation.

All of those sequences obtained from *B. recurrentis* showed identical signature single-nucleotide polymorphisms (SNPs), including the premature stop codon of UAG, with one exception at position 169, where a synonymous SNP divided *B. recurrentis* sequences into two groups. Nine other SNPs were present that differentiated *B. recurrentis* from *B. duttonii* (Table 2).

Although the presence of this premature stop codon cannot categorically rule out expression of RecA, we attempted to demonstrate *recA* expression by reverse transcription analysis, but failed to produce detectable products in patient samples infected with either spirochaete, possibly because of degradation resulting from the prolonged frozen storage of these samples prior to testing, or reflecting insufficient sensitivity (data not shown). If this investigation had used cultivable isolates, it would have been possible to increase the quantity of starting material and thus undertake expression analysis. Analysis of other microbial *recA* mutants has utilized the demonstration of increased susceptibility to UV light; however, cultivation of these fastidious spirochaetes is particularly challenging, thus precluding such investigations. Indeed, the poor success in cultivating these organisms and greater diversity of spirochaetes detected in arthropods and patient samples were the reasons why we decided to analyse the *recA* gene directly within patient samples, thus removing the selective pressures of cultivation [2,3].

The RecA paradigm, based upon its role in *Escherichia coli*, is repair of double-stranded breaks in DNA that occur at stalled replication forks during the process of DNA replication. It also has a significant role following exposure to stress conditions that might result in DNA damage, and thus is considered to have a pivotal role in the SOS response of microorganisms involving *recA*, *lexA* and many other effector genes. No evidence of *lexA* or any orthologues has been reported in either *Borrelia burgdorferi*, the causative agent of Lyme borreliosis, or in the relapsing fever borreliae sequenced to date, suggesting that the SOS response may be absent in these spirochaetes [4]. The role of RecA within *Borrelia* remains to be fully elucidated, but it appears not to

have a key homologous recombination role in VlsE variation within *B. burgdorferi* [4]. RecA mutant strains of *B. burgdorferi* were still able to undergo mosaic VlsE lipoprotein variation, so RecA would appear not to have a role in the recombination events utilized for antigenic variation in *Borrelia*, in contrast to other organisms, such as *Neisseria gonorrhoeae* [5].

Furthermore, possession of non-functional RecA has been shown not to have any deleterious effects on the infectiousness of *B. burgdorferi*. This is in contrast to the lethality of RecA mutations hypothesized by others [6]. However, this strain showed a loss in ability to cause joint infections [4]. These findings suggest that loss of RecA may not affect the viability of the closely related relapsing fever spirochaetes, but could account for differences in clinical consequences. Indeed, clinical differences are seen between these infections, with *B. recurrentis* resulting in significant jaundice, petechiae, epistaxis and major organ involvement (central nervous system and cardiac), and *B. duttonii* being associated with pregnancy complications and high perinatal mortality [7]. The obvious difference between these two spirochaetes is their ability to be either tick-borne or louse-borne, resulting in either local endemic or epidemic disease, respectively. It is unlikely that RecA is not necessary among louse-borne pathogens, as RecA remains functional in *Rickettsia prowazekii*, another louse-borne pathogen whose genome is also subject to degradation as compared with non-louse-borne counterparts [8]. Whether the truncation of RecA within *B. recurrentis* plays a role in the differing clinical presentations or vectorial capabilities of these spirochaetes remains to be determined. What is apparent is that samples from louse-borne relapsing fever patients showed the same 'signature' SNPs, differentiating them from *B. duttonii*, including the premature stop codon, thus suggesting a clonal ancestry.

Acknowledgements

We thank R. Singh for his technical assistance.

Transparency Declaration

The authors declare no conflict of interests.

References

1. Lescot M, Audic S, Robert C *et al.* The genome of *Borrelia recurrentis*, the agent of deadly louse-borne relapsing fever, is a degraded subset of tick-borne *Borrelia duttonii*. *PLoS Genet* 2008; 4: e1000185.
2. Cutler SJ, Bonilla EM, Singh R. Insights into the population structure of East African relapsing fever *Borrelia*. *Emerg Infect Dis* 2010; [Epub ahead of print]. In review.
3. Scott JC, Wright DJM, Cutler SJ. Typing African relapsing fever spirochetes. *Emerg Infect Dis* 2005; 11: 1722–1729.
4. Liveris D, Mulay V, Sandigursky S, Schwartz I. *Borrelia burgdorferi* vlsE antigenic variation is not mediated by RecA. *Infect Immun* 2008; 76: 4009–4018.
5. Helm RA, Seifert HS. Pilin antigenic variation occurs independently of the Recbcd pathway in *Neisseria gonorrhoeae*. *J Bacteriol* 2009; 191: 5613–5621.
6. Putteet-Driver AD, Zhong J, Barbour AG. Transgenic expression of *recA* of the spirochetes *Borrelia burgdorferi* and *Borrelia hermsii* in *Escherichia coli* revealed differences in DNA repair and recombination phenotypes. *J Bacteriol* 2004; 186: 2266–2274.
7. Cutler SJ, Abdissa A, Trape J-F. New concepts for the old challenge of African relapsing fever borreliosis. *Clin Microbiol Infect* 2009; 15: 400–406.
8. Dunkin SM, Wood DO. Isolation and characterization of the *Rickettsia prowazekii recA* gene. *J Bacteriol* 1994; 176: 1777–1781.

Molecular epidemiological investigation of multidrug-resistant *Acinetobacter baumannii* strains in four Mediterranean countries with a multilocus sequence typing scheme

A. Di Popolo[1,*], M. Giannouli[1,*], M. Triassi[1], S. Brisse[2] and R. Zarrilli[1,3]

1) *Dipartimento di Scienze Mediche Preventive, Università di Napoli Federico II, Naples, Italy,* 2) *Institut Pasteur, Genotyping of Pathogens and Public Health, Paris, France and* 3) *CEINGE Biotecnologie Avanzate, Naples, Italy*

Abstract

Thirty-five multidrug-resistant *Acinetobacter baumannii* strains, representative of 28 outbreaks involving 484 patients from 20 hospitals in Greece, Italy, Lebanon and Turkey from 1999 to 2009, were analysed by multilocus sequence typing. Sequence type (ST)2, ST1, ST25, ST78 and ST20 caused 12, four, three, three and two outbreaks involving 227, 93, 62, 62 and 31 patients, respectively. The genes $bla_{oxa\text{-}58}$, $bla_{oxa\text{-}23}$ and $bla_{oxa\text{-}72}$ were found in 27, two and one carbapenem-resistant strain, respectively. In conclusion, *A. baumannii* outbreaks were caused by the spread of a few strains.

Keywords: *Acinetobacter baumannii*, carbapenemases, molecular epidemiology, multilocus sequence typing, pulsed-field gel electrophoresis