Development and validation of loop-mediated isothermal amplification (LAMP) assays for the detection of *Acinetobacter* spp., *Corynebacterium* spp., *Enterobacter* spp. and *Streptococcus canis* recovered from surgical site infections (SSIs) of companion animals

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Introduction

- Loop-Mediated Isothermal Amplification (LAMP)-based assays have been described for important human and animal pathogens.
- Companion animals, especially dogs and cats, are known to carry clinically significant, antibiotic-resistant bacteria with zoonotic potential.
- We describe the development of four novel LAMP-based assays designed to detect commonly encountered, veterinary bacterial pathogens.
- The aim of the work presented here, was to develop robust, sensitive and rapid tests for *Acinetobacter* spp., *Corynebacterium* spp., *Enterobacter* spp. (as well as other important Enterobacteriaceae spp.) and *Streptococcus canis*, respectively.

Materials & Methods

- Both type strains and well characterized clinical isolates (veterinary and human) were used in this study.
- All isolates used were fresh streak plate cultures on appropriate media, incubated aerobically overnight at 37°C.
- Crude bacterial lysates, prepared using single colonies, were used as template in LAMP reactions. DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Southampton, UK) and was used as template to determine the limit of detection (LOD).
- Primers were designed using LAMP Designer software: V1.15 (PREMIER Biosoft, Pardesipura, India).
- LAMP assays were performed using the Genie II Mark I instrument (OptiGene, Horsham, UK), software: V1.28.
- The GspSSD Isothermal Master Mix ISO-001 (OptiGene) was used and the primer concentrations were as suggested by the manufacturer.
- Standard reaction conditions were as follows: amplification, 30 minutes at 65°C; anneal, 98°C - 80°C (ramp rate: 0.1°C per minute).

Results & Discussion

- The *Acinetobacter* spp. assay, designed to target a Tex-like transcriptional accessory factor-encoding gene, reproducibly detected *A. baumannii* ATCC 19606 and clinical isolates belonging to PFGE-defined, epidemic UK clones, including isolate AB16 (representative of 'OXA-23 clone 2'; Table 1). Primer sequences (5' to 3'): AB_F3, CTGGTATTCGTACTGGTGTG; AB_B3, TGATATTGACCTACGCCAATC; AB_FIP, ACGGCTCGCAGTACCATTGAGAAGGCTCAATTGCTGAG; AB_BIP, GCGTAACCGTAAGTGAAGCAGGACTGCACCACGGATTGAA; AB_LoopF, GCTCGACATTGTATTCACGAC; AB_LoopB, GGAACTGCCAGAGCTTGAT.

Table 1. Biological replicates of *A. baumannii* isolates, ATCC 19606 & AB16.

Isolate ID	Anneal temperature (°C)			
	Biological replicate 1	Biological replicate 2	Biological replicate 3	Mean (+/- SD)
ATCC 19606	86.54	86.73	86.76	**86.68 (0.12)**
AB16	86.64	86.78	86.76	**86.73 (0.08)**
	Amplification time (mins)			
	Biological replicate 1	Biological replicate 2	Biological replicate 3	Mean (+/- SD)
ATCC 19606	7.50	6.75	7.00	**7.58 (0.38)**
AB16	**7.75**	**7.50**	**7.50**	**7.58 (0.14)**

- The type strains *C. pseudotuberculosis* NCTC 3450 and *C. ulcerans* NCTC 7910, representing important veterinary pathogens, as well as an additional veterinary *Corynebacterium* spp. isolate, were detected using a *rpoB*-based assay (Table 2). Primer sequences (5' to 3'): C_rpoB_F3, GCGTGAGGTGCTGGAAG; C_rpoB_B3, TGCTGACGAGCACGCC; C_rpoB_FIP, GAAAAACGTTCGGGAGCCCCGGACCCATCTTGGCAGTCTC; C_rpoB_BIP, ACGGAACCTATTGAGGTCCCGGATGAGCCATGCGAAGGAATC; C_rpoB_LF, GGCCACTGACTTGGTCTGG.

Table 2. Biological replicates of *C. pseudotuberculosis* NCTC 3450 *C. ulcerans* NCTC 7910.

Isolate ID	Anneal temperature (°C)			
	Biological replicate 1	Biological replicate 2	Biological replicate 3	Mean (+/- SD)
NCTC 3450	89.30	89.40	89.50	**89.40 (0.10)**
NCTC 7910	89.50	89.50	89.50	**89.50 (0.00)**
	Amplification time (mins)			
	Biological replicate 1	Biological replicate 2	Biological replicate 3	Mean (+/- SD)
NCTC 3450	6.00	6.00	6.00	**6.00 (0.00)**
NCTC 7910	7.50	7.50	7.25	**7.42 (0.14)**

- The *Enterobacter* spp. assay, targeting *rpoB* could identify the most clinically relevant species, *E. cloacae* (Table 3) among others. Primer sequences (5' to 3'): E2_F3, CAACCCGCTGTCTGAGAT; E2_B3, TCCATGTAGTCAACCTGGT; E2_FIP, ATGTTTGGACCTTCAGGCGTTGAAGTTCGAGACGTACACC; E2_BIP, GAAGAAGGCAACTACGTTATCGCAAGTGGCCTTCGTCATCC; E2_LoopF, ATTGGACATACGCGACCG; E2_LoopB, TCAGGCGAACTCCAACCT.

Table 3. Technical replicates replicates of *E. cloacae* NCTC 13380.

Isolate ID:	Anneal temperature (°C)		
	Technical replicate 1	Technical replicate 2	Mean (+/- SD)
NCTC 13380	87.20	87.20	**87.20 (0.00)**
	Amplification time (mins)		
	8.25	**8.25**	**8.25 (0.00)**

- A species-specific LAMP assay was designed to detect *S. canis*, based on the CAMP factor-encoding gene of sequenced isolate, FSL S3-227. The inter-assay coefficient of variation (CV) was 0.07% for *S. canis* NCTC 12191 (Table 4) and the intra-assay CV in the example experiment presented in Figure 1 was 0.08% for all isolates tested. The LOD was established and was found to be 0.4 pg/µL of extracted DNA. No cross-reactivity was observed, even with closely related species belonging to Lancefield groups A, C and G, among other organisms. Primer sequences (5' to 3'): SC_F3, TTCCAACCGTAGGTCAGTC; SC_B3, ATGGCATCAACTGATGTGAA; SC_FIP, GCATCAGTGCCTTTAACCTCGTCCCTGATGCTGTCTTAGCTGATA; SC_BIP, GTTCGTTTCAGCCCTGATACCATCGCTTGAATAGCTTCTGACAATG; SC_LoopF, TTGAGCAGTCACTTGATTGT; SC_LoopB, TCAATTGGTGCAAGAGTTGAAG.

Table 4. Biological replicates of *S. canis* NCTC 12191.

Isolate ID:	Anneal temperature (°C)			
	Biological replicate 1	Biological replicate 2	Biological replicate 3	Mean (+/- SD)
NCTC 12191	84.5	84.57	84.45	**84.51 (0.06)**
	Amplification time (mins)			
	12.5	11.75	10.75	**11.67 (0.88)**

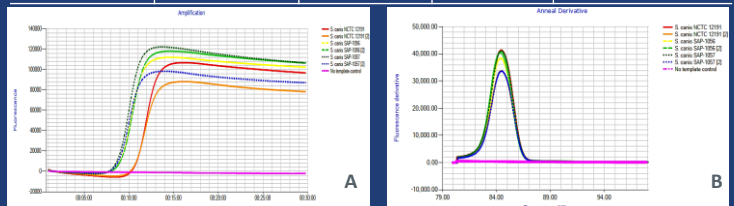

Figure 1. Amplification curves (A) & anneal peaks (B) of *S. canis* NCTC 12191 & veterinary isolates, SAP-1056 and SAP-1057.

Conclusions

- **All assays reproducibly detected both characterized type strains and uncharacterized isolates of clinical (veterinary and/or human) origin *in vitro* in less than 30 minutes.**
- **Full scale validation of all assays is currently in progress and data presented here indicates their potential clinical utility for both medial and veterinary use.**

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