Institut für Medizinische Mikrobiologie, Virologie und Hygiene

Bone Innovation Summit, 13.02.2019

Diagnostic approaches in implant associated-infections

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Diagnostic workflow in PJI: the ideal world

Working hypothesis: PJI

Clinical consequences

< 20 h

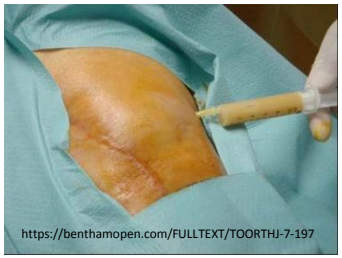
Targeted therapy

Communication

Interpretation
- ABS
- Expert system

Emperic anti-infective therapy

Specimen collection

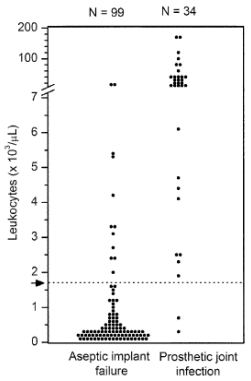


Microbiological toolbox

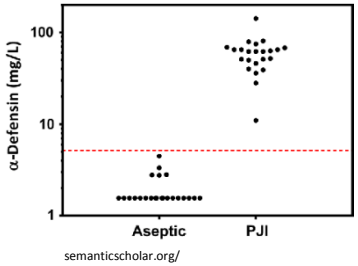
Detection
Differentiation
Susceptibility testing

Invasive PJI diagnostics: current concepts and approaches

Biomarkers



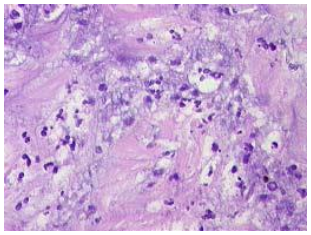
> 1,7 x 10^3 PMN/µl



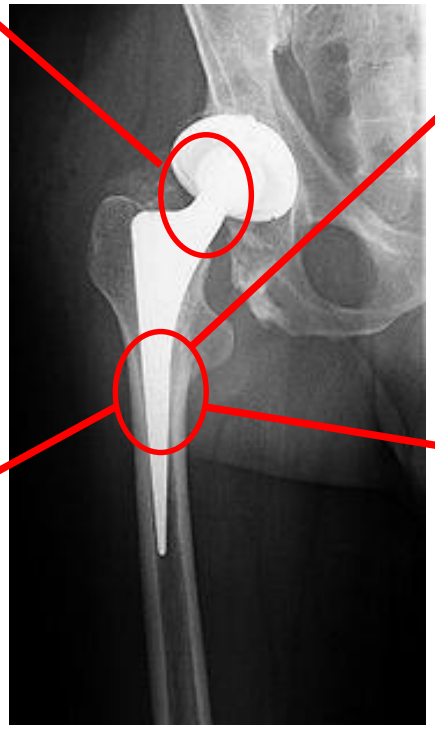
semanticscholar.org/

α-Defensin positive

Histo-pathology



> 5 PMN / 10 HPF



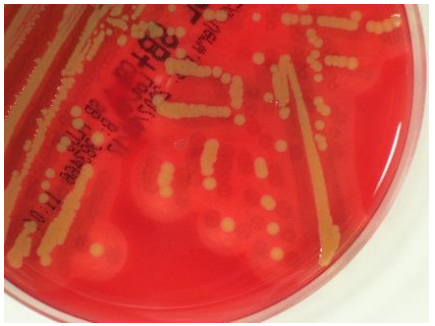
Trampuz, Zimmerli, 2004

Sia, Berbari, Karchmer, 2005

Osmon et al., CID 2013

Microbiology

Culture



Culture-independent: PCR

https://online-shop.eppendorf.de/DE-de/PCR-44553/Cycler-44554/Mastercycler-pro-PF-5193.html

Pathogen epidemiology in PJI

TABLE 1 Common causes of prosthetic joint infection

	% of patients with prosthetic joint infection					
	Hip and knee					
Infection	All time periods[a]	Early infection[b]	Hip[c]	Knee[c]	Shoulder[d]	Elbow[e]
Staphylococcus aureus	27	38	13	23	18	42
Coagulase-negative *Staphylococcus*	27	22	30	23	41	41
Streptococcus species	8	4	6	6	4	4
Enterococcus species	3	10	2	2	3	0
Aerobic Gram-negative bacilli	9	24	7	5	10	7
Anaerobic bacteria	4	3	9	5		
Propionibacterium acnes					24	1
Other anaerobes					3	0
Culture negative	14	10	7	11	15	5
Polymicrobial	15	31	14	12	16	3
Other	3					

Tande, Patel, CMR 2014

- **Infections caused by commensal bacteria from skin microbiota.**
- **Significant proportion of infections remain etiologically unresolved.**
- **Polymicrobial infections possible / more common than anticipated.**

Diagnostic challenges in PJI

- **Culture-negative PJI:** Advanced diagnostics (culture-independent).
- **Polymicrobial infections:** Discrimination of complex microbial consortia.
- **Skin commensals:** Differentiation between contamination and invasive isolates.

Molecular approaches to PJI diagnosis: targeted amplification or broad range PCR

Species-specific PCR

Primer for

- ***S. aureus***
- ***Staphylococcus sp.***
- ***Enterococcus sp.***
- ***Streptococcus sp.***
- ***C. acnes***
- **Enterbacteriaceae**

Pro

- **(sensitivity)**
- **Speed (signal = diagnosis)**

Con

- **Species not included in the panel**

Pan-bacterial PCR

Pan-bacterial primer:

Pro

- **Universal detection of (all) bacterial species**

Con

- **Sensitivity**
- **Specificity (polymicrobial infections)**
- **Speed (need for sequencing)**

Broad range PCR detection of bacterial pathogens: supporting evidence

Detection of pathogens in explanted heart valves

137 episodes (172 samples): PCR and culture from explanted valve material

	PCR positive	PCR negative
Culture positive	22	77
Culture negative	38	

Viridans streptococci	8
Cutibacterium species	3
Aggregatibacter actinomycetemcomitans	2
Bartonella species	2
CoNS	2
Staphylococcus aureus	2
Streptococcus agalactiae	2
Tropheryma whipplei	1
unknown	1

Christner, Rohde, in preparation

Molecular diagnostics in PJI: unresolved problems

Specimens:

- 434 samples
- 144 infections
- 290 aseptic loosening

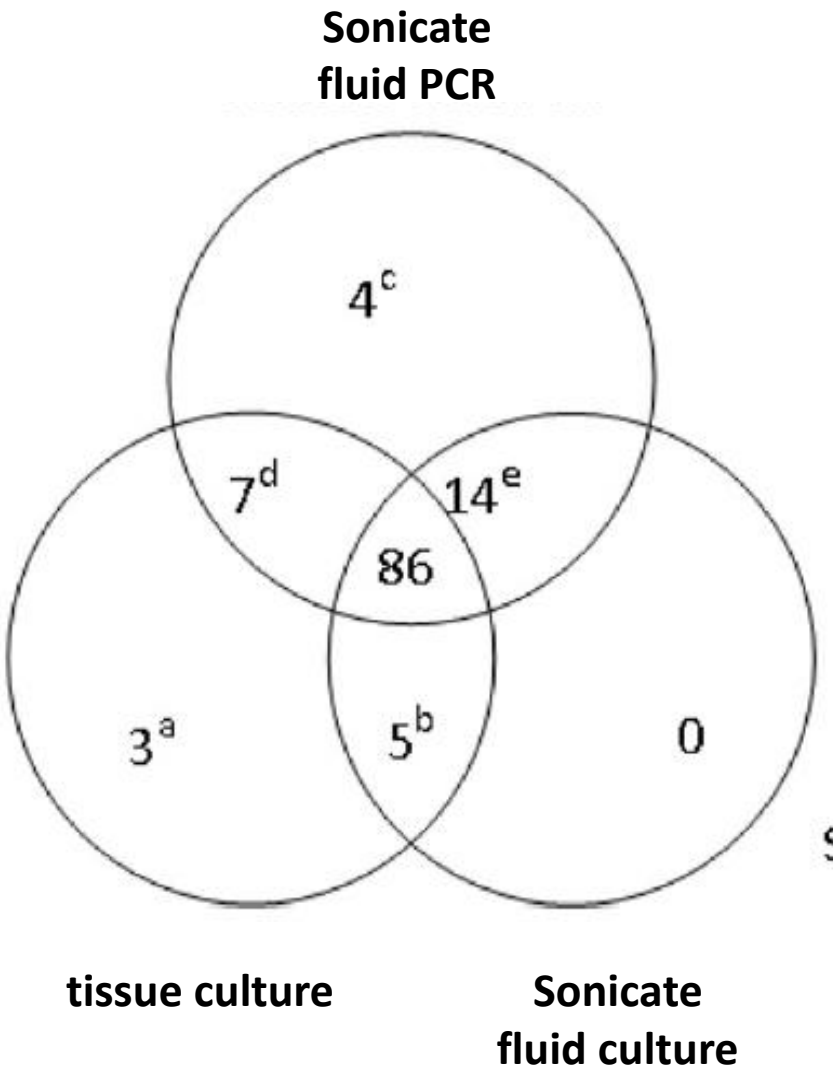
Method:

- Multiplex PCR Panel (10 most common pathogens) vs. conventional culture

Result

- PCR sensitivity: 77.1 %

BUT: discordant results possible



Cazanave et al., J. Clin. Microbiol 2013

Molecular diagnostics in PJI: detection limit

	Number of colonies		
	<5	5–50	>50
	Available results, $n = 94$		
Culture and mPCR positive concordant results $n = 102$ (%)	13 (13.8)	32 (34.0)	49 (52.2)
	Available results, $n = 65$		
Culture positive and mPCR negative results $n = 115$ (%)	30 (39.4)	21 (37.9)	14 (22.7)

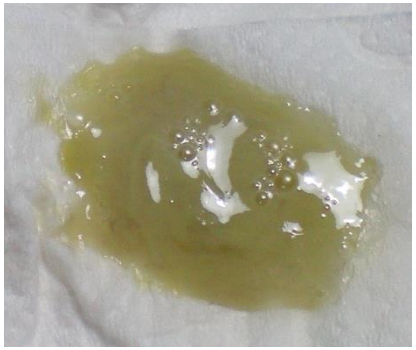
Malandain et al., CMI 2018

Traditional diagnostic approaches in infectious diseases approaches are inherent restricted to detect known pathogens.

Metagenomic sequencing represents a hypothesis-free methodology allowing to detct the „unknown unknowns“.

Identification of unknown pathogens using metagenomics shotgun sequencing

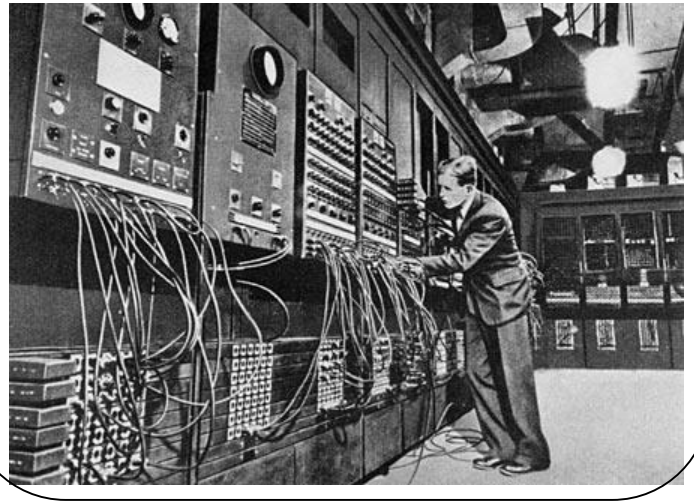
Patient specimen



DNA extraction
human / viral / bacterial

Sequencing

Bioinfomatics



Pathogen A

Pathogen B

Pathogen C

Pathogen D

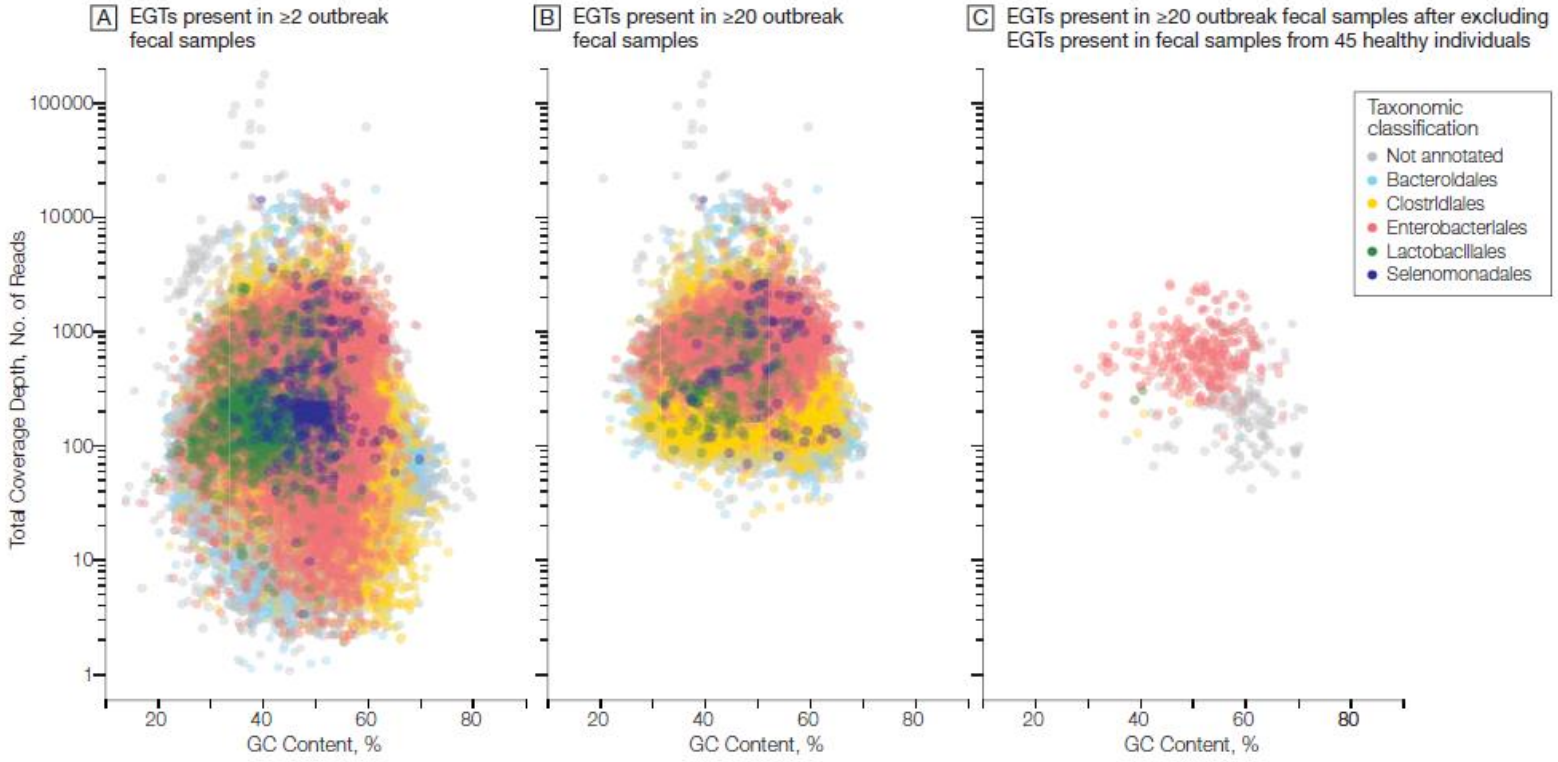
Pathogen E

etc. ……

Metagenomics allows for culture-free detection and genome reconstruction directly from clinical samples

Sequences in ≥ 2 samples **Sequences in ≥ 20 samples** **Sequences in ≥ 20 samples, Sequences from healthy subtracted**

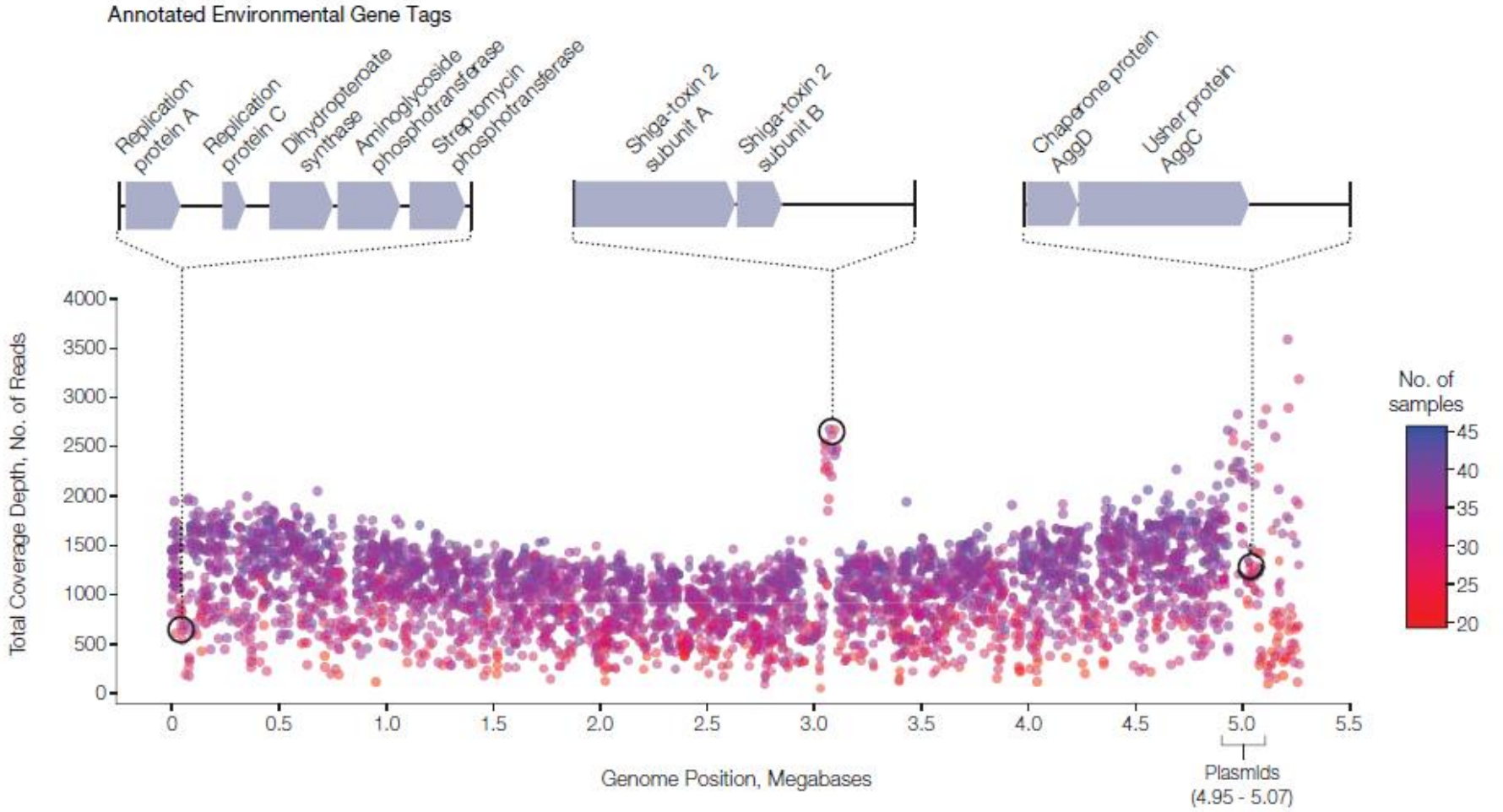
Figure 2. Recovery of Sequences From the Outbreak Strain From the Outbreak Metagenome Through Iterative Filtering



Each point on the scatter plot shows the GC content (x-axis) and total depth of coverage (y-axis, $\log_{10}$-scale) colored by taxon for each environmental gene tag (EGT) in the outbreak metagenome. Numerical values for the EGTs presented in each panel are available in the eSupplement at http://www.jama.com.

Loman, Christner, Rohde et al., JAMA 2013
Rohde et al., NEJM 2011

Metagenomics allows for culture-free detection and genome reconstruction directly from clinical samples



Loman, Christner, Rohde et al., JAMA 2013
Rohde et al., NEJM 2011

Metagenomic detection of pathogens and prediction of antibiotic susceptibilities from urine

Urine and species	Method[a]	Penicillins and inhibitor combinations			Cephalosporins, monobactams and inhibitor combinations								Fluoroquinolone	Aminoglycosides				Antifolate
		AMP	AMC	TZP	CTX	CTX/CLO	CTX/CLA	CAZ	CAZ/CLA	FEP	FEP/CLA	FOX	AZT	CIP	AMK	TOB	GEN	STR	TMP
CU5 *K. pneumoniae*	MICs	>64	16	8	128	64	≤0.06	16	0.25	8	≤0.06	4	16	2	2	16	32	R	R
	MinION		bla_{OXA-1}					$bla_{CTX-M-15}$, bla_{SHV-32}						*qnrB*, *aac(6')-Ib-cr*		*aac(6')-Ib-cr*, *aacC2*, *strA*			*dfrA14*
	Illumina		bla_{TEM-1}, bla_{OXA-1}					$bla_{CTX-M-15}$, bla_{SHV-27}, bla_{LEN-12}						*qnrB*, *aac(6')-Ib-cr*		*aac(6')-Ib-cr*, *aacC2*, *strA*, *strB*			*dfrA14*
CU6 *E. coli*	MICs	>64	16	4	128	32	≤0.06	16	0.25	8	≤0.06	8	32	>8	4	16	16	S	R
	MinION		$bla_{TEM\ (mv)}$, bla_{OXA-1}				$bla_{CTX-M\ gp1\ (15)}$, *ampC* ($bla_{CMY\ mv}$, bla_{ACC-4}, bla_{MIR-9}, bla_{DHA-22})							*aac(6')-Ib-cr*		*aac(6')-Ib-cr*, *aacC2*, *aadA5*			*dfrA17*
	Illumina		bla_{TEM-1}, bla_{OXA-1}					$bla_{CTX-M-15}$						*aac(6')-Ib-cr*, *gyrA* (83:SL; 87:D-N), *parC* (80:S-I; 84:E-V)		*aac(6')-Ib-cr*, *aacC2*, *aadA5*			*dfrA17*
CU7 *E. coli*	MICs	>64	8	2	128	32	≤0.06	8	0.12	4	≤0.06	4	16	0.25	2	1	0.5	R	R
	MinION		$bla_{TEM\ (mv)}$					$bla_{CTX-M\ gr1}$, *ampC* (bla_{ACT-24})								*aadA1*, *aadA3*, *strA*, *strB*			*dfrA1*
	Illumina		bla_{TEM-1}					$bla_{CTX-M-15}$						*gyrA* (83:S-L)		*aadA1*, *strA*, *strB*			*dfrA1*
CU8 *E. coli*	MICs	64	32	4	1	≤0.12	0.25	0.5	0.5	≤0.12	0.12	>64	0.25	≤0.12	1	0.5	0.5	S	S
	MinION						*ampC* ($bla_{CMY\ mv}$, bla_{ACC-4}, bla_{MIR-4}, bla_{DHA-6}, bla_{FOX4})												
	Illumina																		
CU9 *E. cloacae*	MICs	>64	64	4	2	≤0.12	2	1	1	≤0.12	0.12	>64	0.25	≤0.12	1	0.5	0.5	S	S
	MinION							*ampC* ($bla_{CMY\ mv}$, $bla_{ACT-18,\ 24}$)											
	Illumina							*ampC* (bla_{ACT-24})											
CU10 *K. pneumoniae*	MICs	>64	32	>64	>256	256	0.125	128	1	64	≤0.06	16	>64	>8	8	>32	>32	R	R
	MinION		$bla_{TEM\ (mv)}$, bla_{OXA-1}					$bla_{CTX-M\ gr1}$, $bla_{SHV(mv)}$						*aac(6')-Ib-cr*, *qnrB*		*aac(6')-Ib-cr*, *aacA4*, *aacC2*, *aadA3*, *strA*, *strB*			*dfrA14*
	Illumina		bla_{TEM-1}, bla_{OXA-1}					$bla_{CTX-M-15}$, bla_{SHV-28}, bla_{LEN-12}						*gyrA* (83:S-I), *parC* (80:S-I), *aac(6')-Ib-cr*, *qnrB*		*aac(6')-Ib-cr*, *aacC2*, *strA*, *strB*			*dfrA14*

Schmidt et al., JAC 2016

Metagenomic pathogen detection in PJI

- **Metagenomic sequencing from sonic fluid.**
- **Implementation of cut-offs to identify false-positives.**
- **Sensitivity compared to sonic fluid culture:**
  - Species-level: 61/69 (88%)
  - Genus-level: 64/69 (93%)

Street et al., JCM 2017

TABLE 3 Performance of metagenomic sequencing compared to that of synovial fluid culture

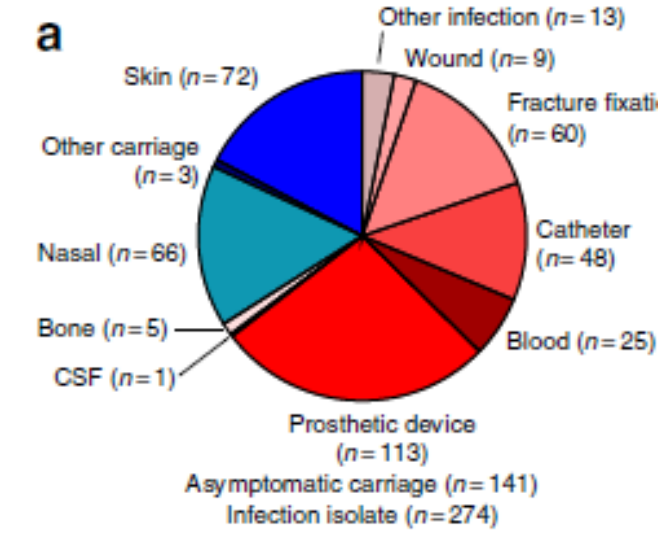
Sample type (*n*)[a]	No. of samples (%)[b]		
	Identical finding	Organism not identified by metagenomics	New organism(s) detected by metagenomics
Aseptic failure (61)	56 (91.8)	1 (1.6)	4 (6.6)
Synovial fluid culture-positive PJI (82)	67 (81.7)	14 (17.1)	3 (3.7)
Synovial fluid culture-negative PJI (25)	21 (84.0)	NA	4 (16.0)

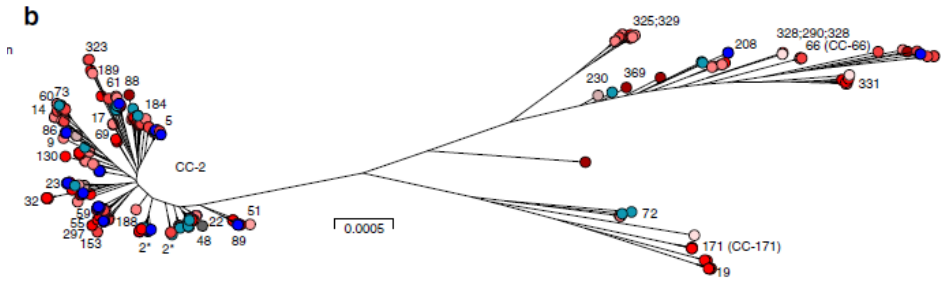
[a]Included are samples for which identical or discrepant findings between synovial fluid culture and metagenomic sequencing were observed. *n*, number of samples in the group.

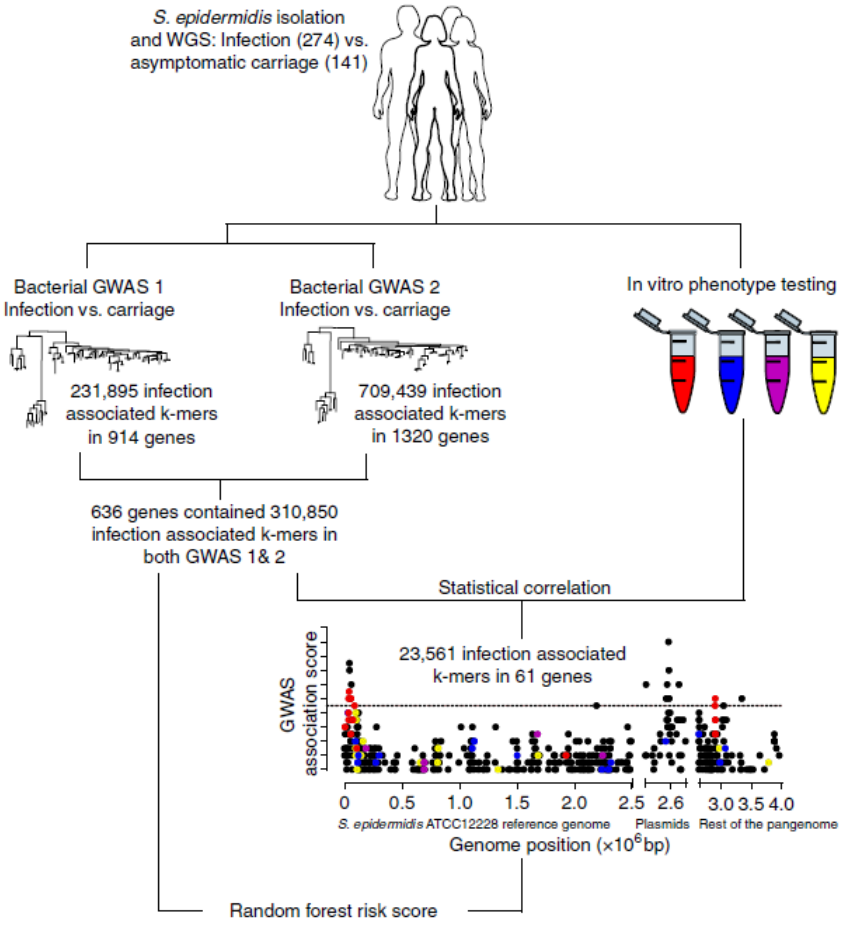
[b]In two cases of culture-positive PJI, the pathogen identified by metagenomics did not match the pathogen identified by synovial fluid culture. These cases are included in the totals shown for both the organisms not identified and the new organisms. NA, not applicable.

Ivy et al., JCM 2018

S. epidermidis populations: evidence for selection of pathogenic clones?



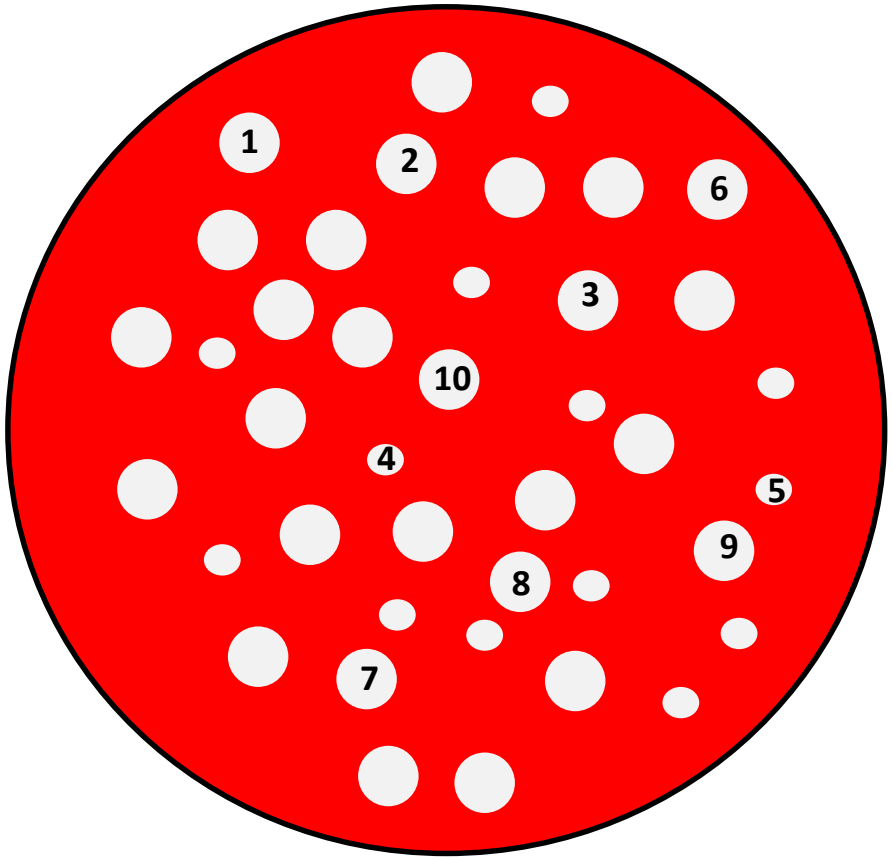




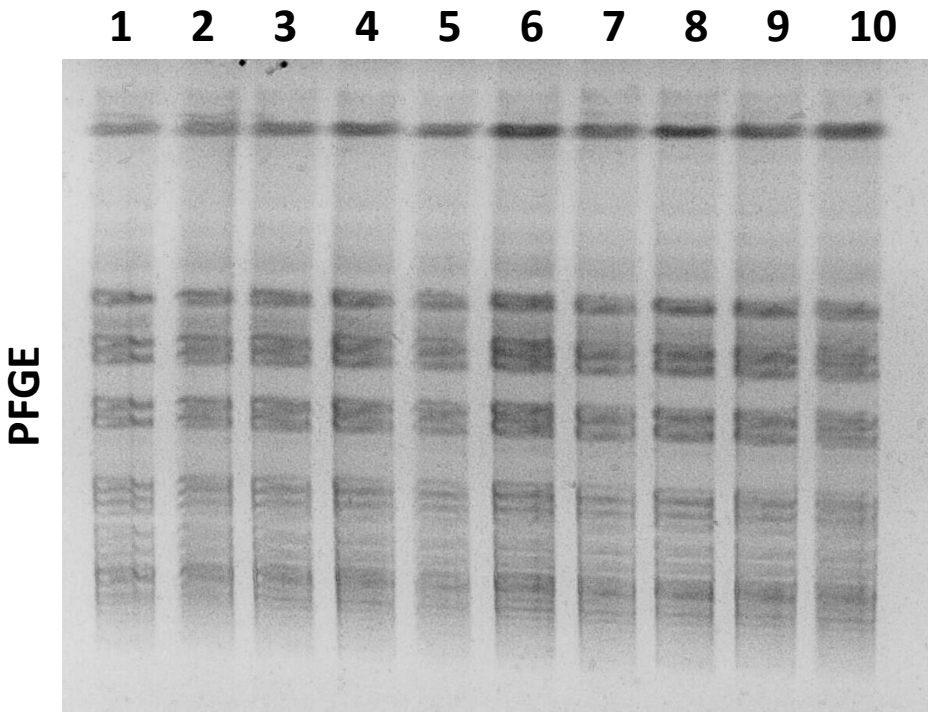
Meric, .., Rohde, Sheppard et al., Nature Communications 2018

S. epidermidis population heterogenity *in vivo*

Primary culture from tissues (PJI)

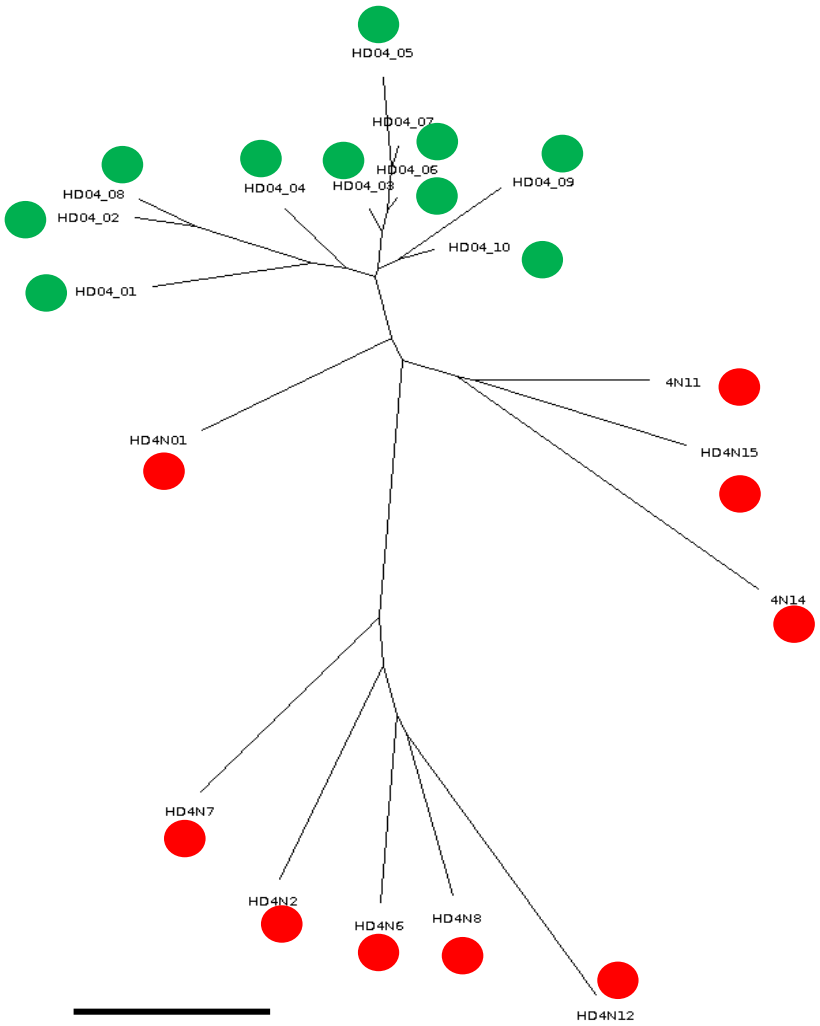


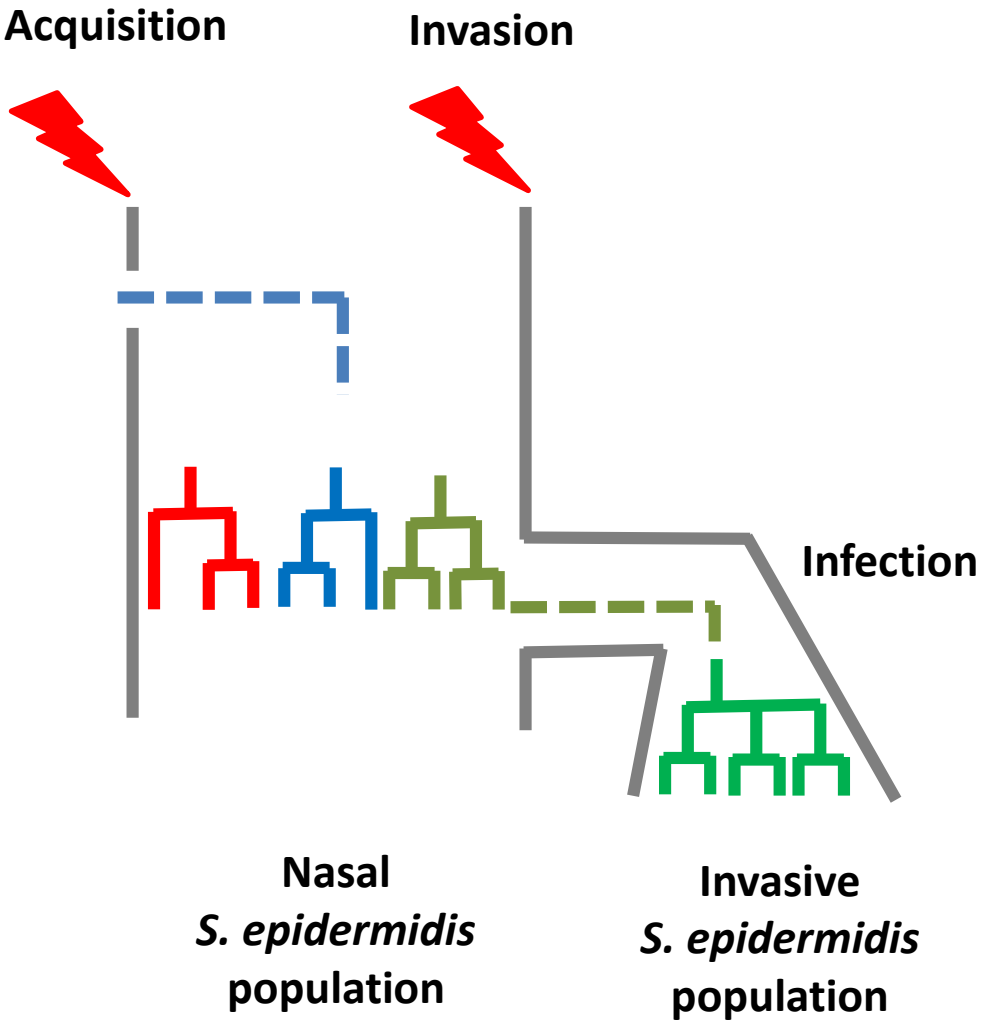
Invasive *S. epidermidis* population



Both, Rohde et al., in preparation

S. epidermidis population heterogenity *in vivo:* genomic plasticity and adaptation





Both, Huang, Rohde, in preparation

Summary

- **Pathogen detection in PJI:** Culture-based diagnostic remains gold standard.

- **Molecular detection of pathogens:** of importance in culture negative cases (e.g. earlier antibiotics therapy). Improvement of sample preparation and standardization necessary.

- **Metagenomik:** unbiased pathogen detection. Potential of molecular pathogen analysis (pathogenicity, resistance). Significant number of unclear aspects (e.g. pre-analytic, sample preparation, bioinformatics, costs).

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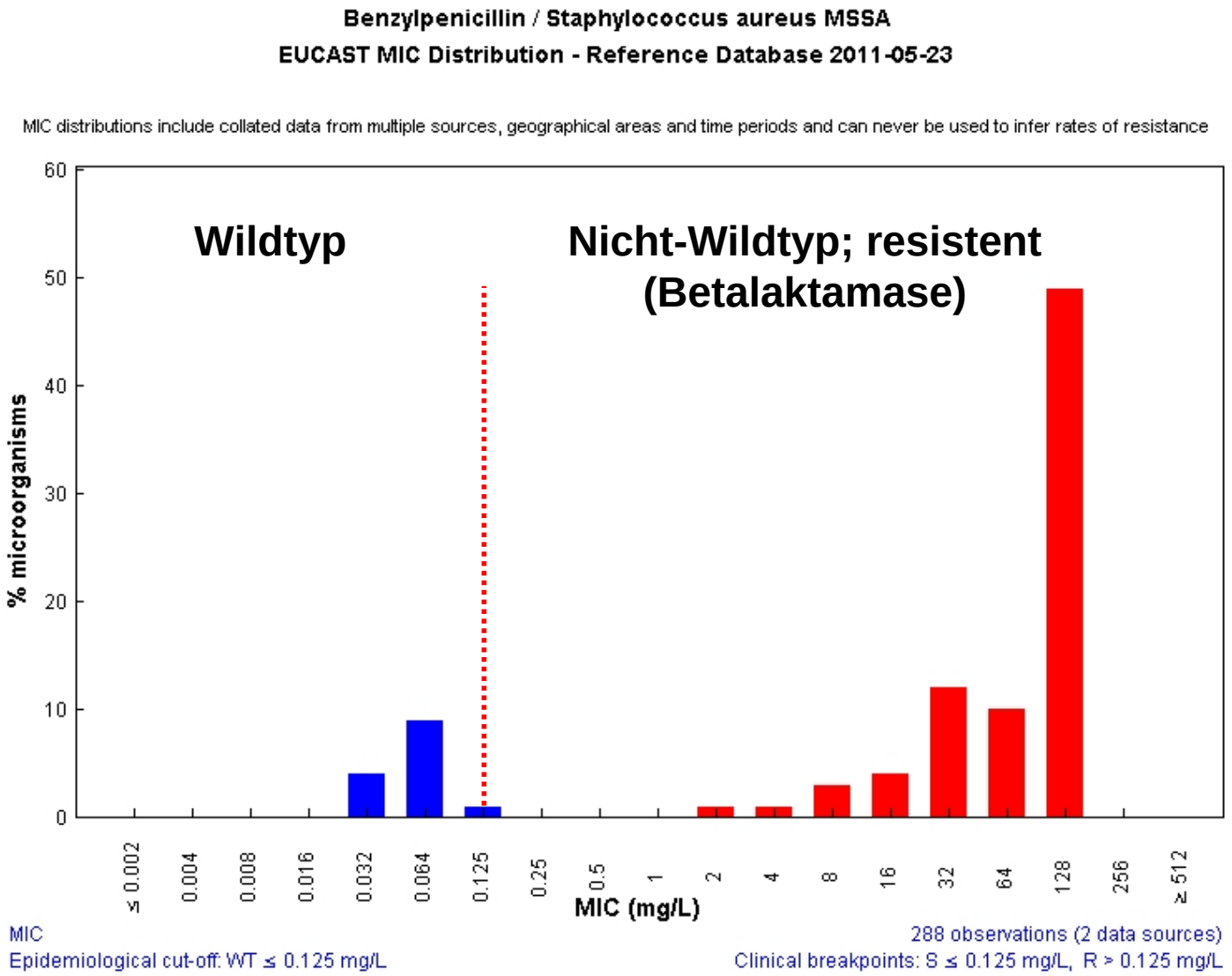
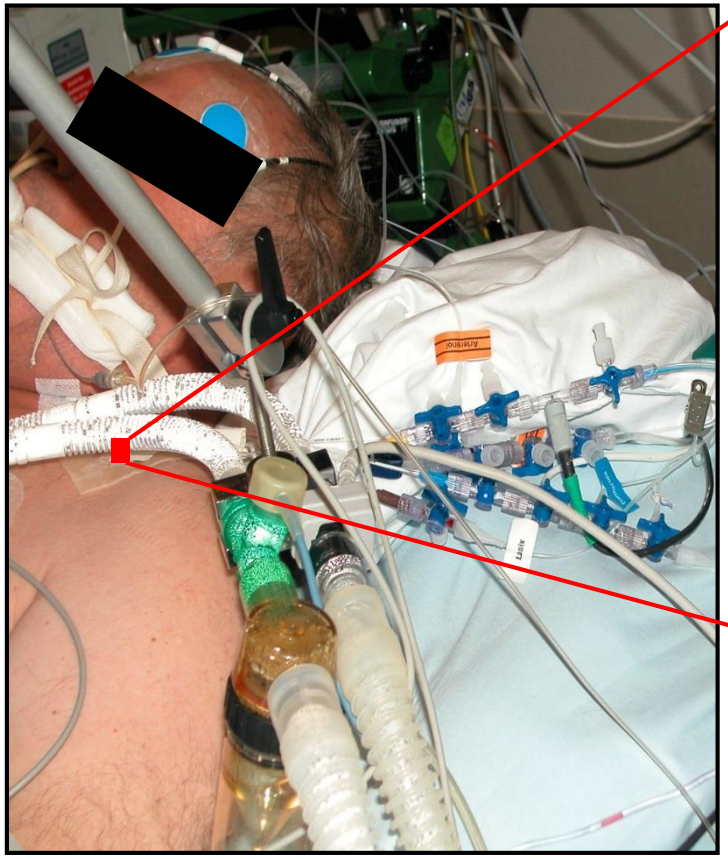
Pfizer

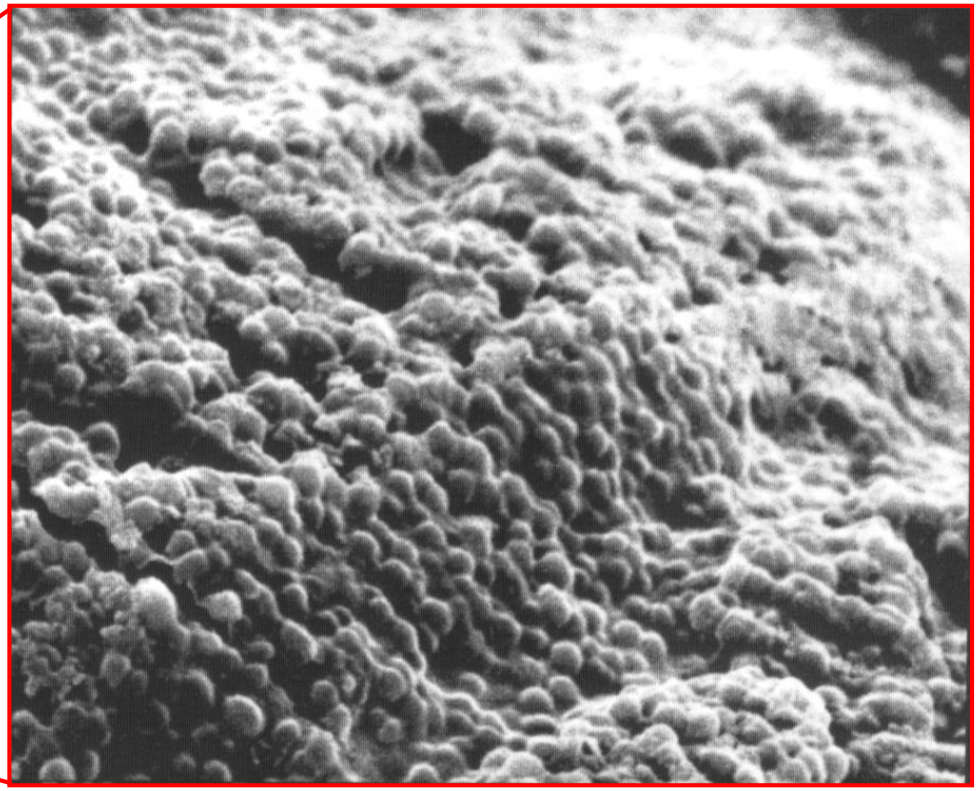
Vorhersage eines Therapieerfolgs durch standardisierte Empfindlichkeitsprüfung



Vorhersage eines Therapieerfolgs durch standardisierte Empfindlichkeitsprüfung

Biofilmbildung ist das wesentliche Merkmal bakterieller Physiologie bei PJI





Metagenomische Detektion von Erregern bei PJI

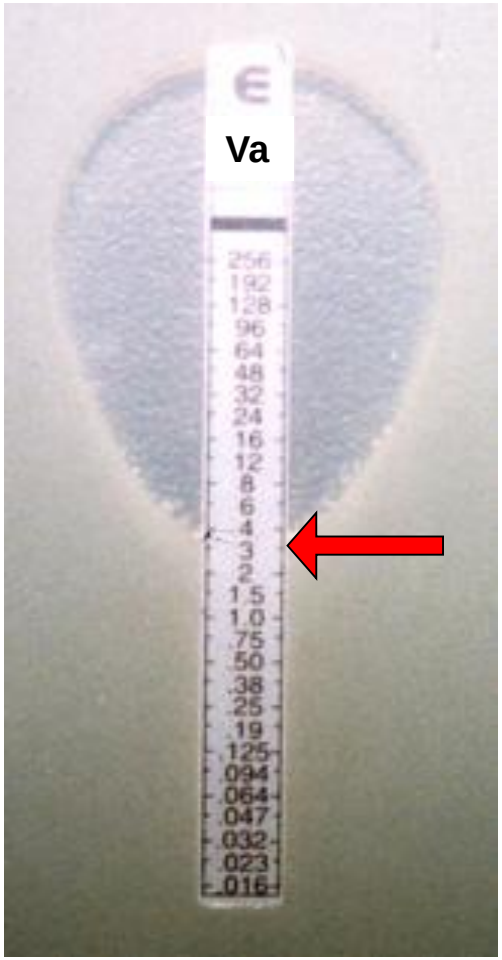
TABLE 1 List of contaminant genera detected in negative controls

Read count	Genus[a]	Previous report(s)[b]
2,017,563	*Acinetobacter*	29, 42, 44, 45
542,456	*Alishewanella*	
386,476	*Ralstonia*	29, 31, 45, 46
186,721	*Anaerococcus*	26, 42
100,847	*Haemophilus*	42
23,008	*Malassezia*	42
16,734	*Enhydrobacter*	29
14,034	*Sphingomonas*	29, 31, 45
3,705	*Paenibacillus*	
3,338	*Delftia*	29, 42
3,179	*Corynebacterium*	25, 29, 42
2,669	*Cutibacterium*	25, 26, 29, 42
	Bradyrhizobium[c]	29, 31, 32

Ivy et al., JCM 2018

Biofilmbildung als Ursache phänotypischer Resistenz

MHK unter Standardbedingungen

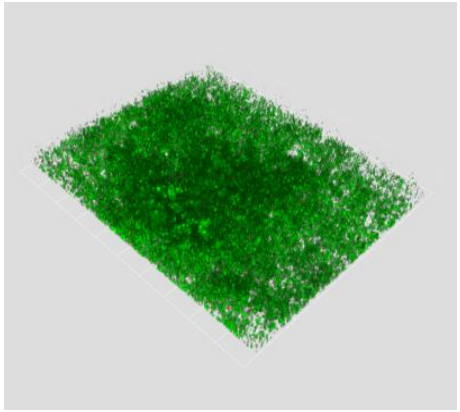


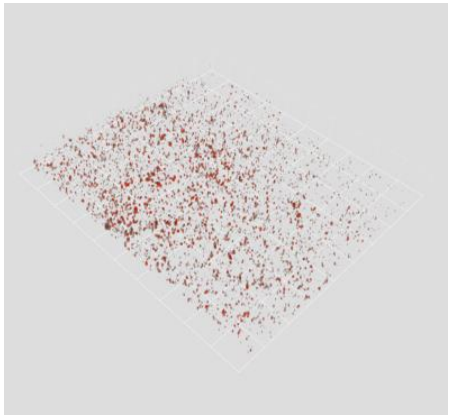
Abtötung von Biofilm-bildenden Bakterien

***S. epidermidis* (lebend)**

***S. epidermidis* (tot)**

Vancomycin: 64 µg/ml

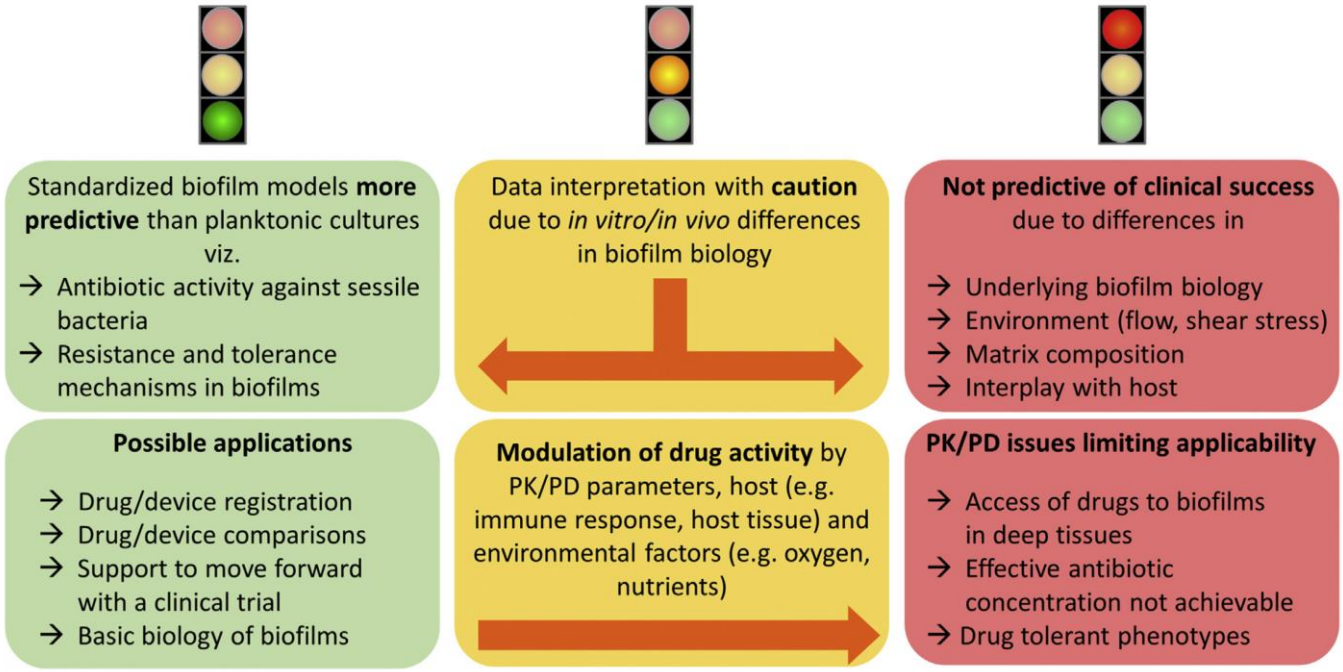




Grün: Lebend-Farbstoff

Rot: Tot-Farbstoff

Aufbau von Systemen zur Beschreibung der Empfindlichkeit biofilmbildender Erreger



Coenye et al., CMI 2018

Es fehlen:

- **Geeignete durchsatzfähige Systeme zur Bestimmung der Empfindlichkeit in Biofilmen.**
- **systematische Daten zur Beschreibung der differentiellen Empfindlichkeit an definierten Patientenisolaten.**
- **Prospektive, klinische Daten.**

Neue Verfahren zur Empfindlichkeitsprüfung bakterieller Erreger

Durchflusszytometrie
Huang et al., Anal Chem 2015

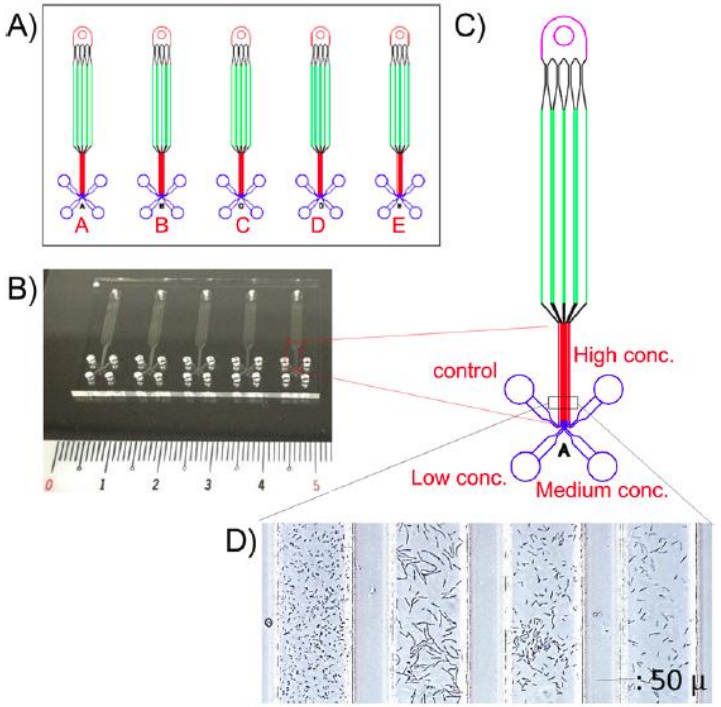
Mikrofluidik
Matsumoto et al., PLoS One 2016

Nanoscale Kulturen
Weibull et al., JCM 2014

Smarticles: lebende Bakterien emittieren Licht
Roche Diagnostics

High resolution imaging
Accelerate Diagnostics

Mikrofluidik: Bakterielles Wachstum in AB-Gradienten



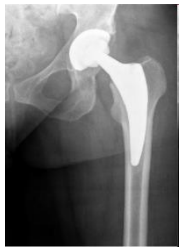
Matsumoto et al., PLoS One 2016

Zusammenfassung

- **Empfindlichkeitsprüfung:** **Methoden zur sicheren Vorhersage von Resistenzphänotypen im Biofilm fehlen. Durchsatzfähigkeit und Möglichkeit zur Testung von Synergismen sollten Ziele technischer Innovation sein.**
- **Derzeit sind keine Daten zur klinischen Nutzbarkeit verfügbar: Es mangelt an zuverlässigen prospektiven Datensätzen.**

Aufbau einer Kohorte “Prosthetic device infections”

Hamburg prosthetic device infection cohort study (HAPDICS)



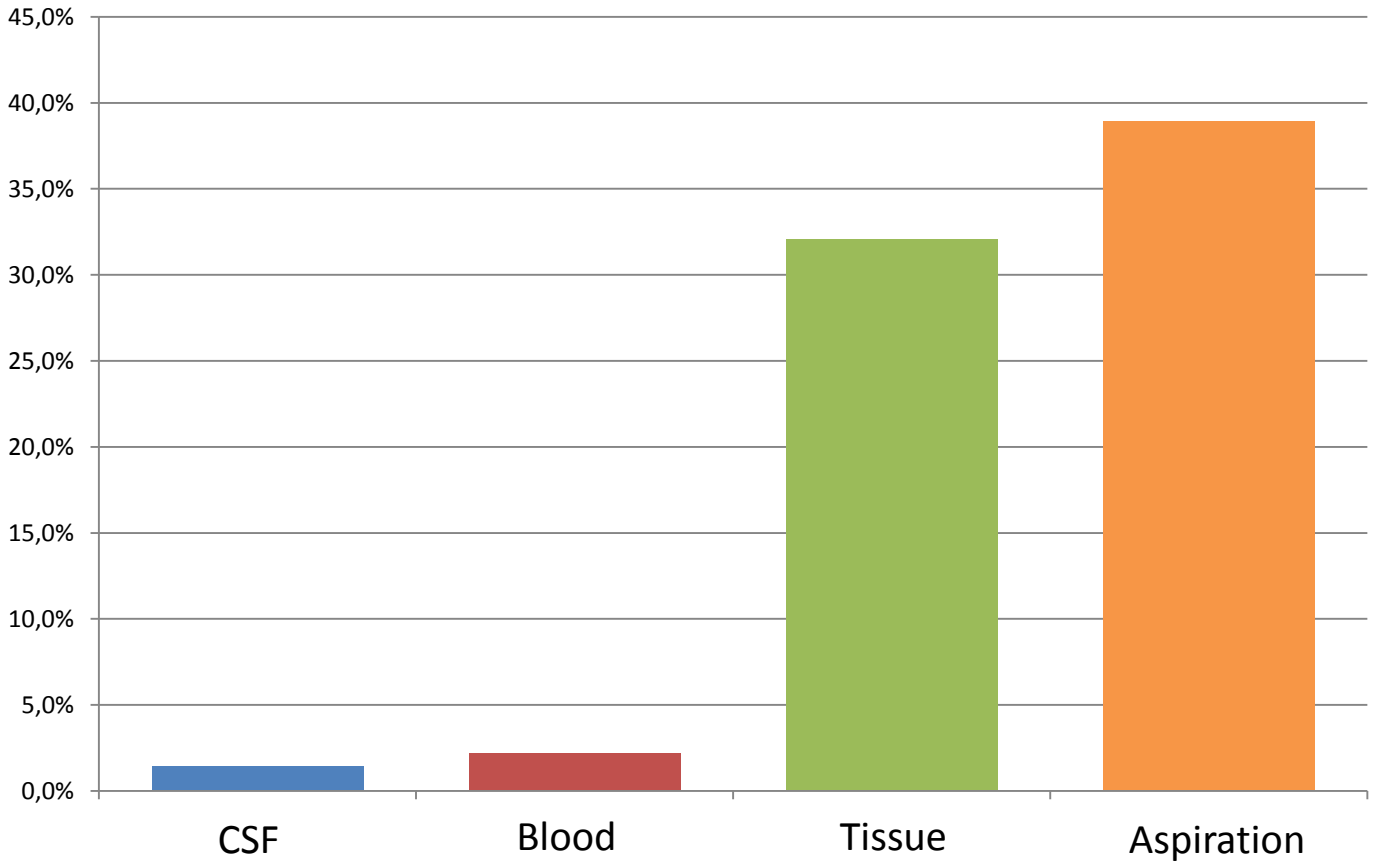
Procedure	Material	Fixation	Processing	Archive
Pre-OP nose swab	Colonizing strain(s)	4 °C	Culture mRNA preparation	Colonizing strain bacterial cDNA
Pre- / intra- OP puncture	Aspirate	4 °C	Quantitative culture	Infecting strain (pre-OP)
Intra-OP Debridement	Tissue / exudate / pus	Fixierung, -20 °C	mRNA preparation	bacterial cDNA
	Tissue/ exudat / pus	4 °C	Quantitative culture	Infecting strain (intra-OP)
			DNA preparation	Whole gDNA
			Histology	Paraffin block

Klinik (ENDO-Klinik, UKE) → Labor (Microbiologie, Pathologie)

DAMP Stiftung 2014 – 2019

Broad range detection of bacterial pathogens: real world experience

Positivity rate 16S-PCR [specimen]



Broad range detection of bacterial pathogens: a positive experience

Detection of pathogens in explanted heart valves

137 episodes (172 samples): PCR and culture from explanted valve material

	PCR positive	PCR negative
Culture positive	22	77
Culture negative	38	

Christner, Rohde, in preparation

Broad range detection of bacterial pathogens: a positive experience

Detection of pathogens in explanted heart valves

137 episodes (172 samples): PCR and culture from explanted valve material

	PCR positive
Culture positive	22
Culture negative	38

Viridans streptococci	5
Enterococcus faecalis	5
Staphylococcus epidermidis	5
Cutibacterium acnes	2
Staphylococcus aureus	2
Corynebacterium diphtheriae	1
Gemella species	1
Staphylococcus lugdunensis	1

Christner, Rohde, in preparation

Broad range detection of bacterial pathogens: a positive experience

Detection of pathogens in explanted heart valves

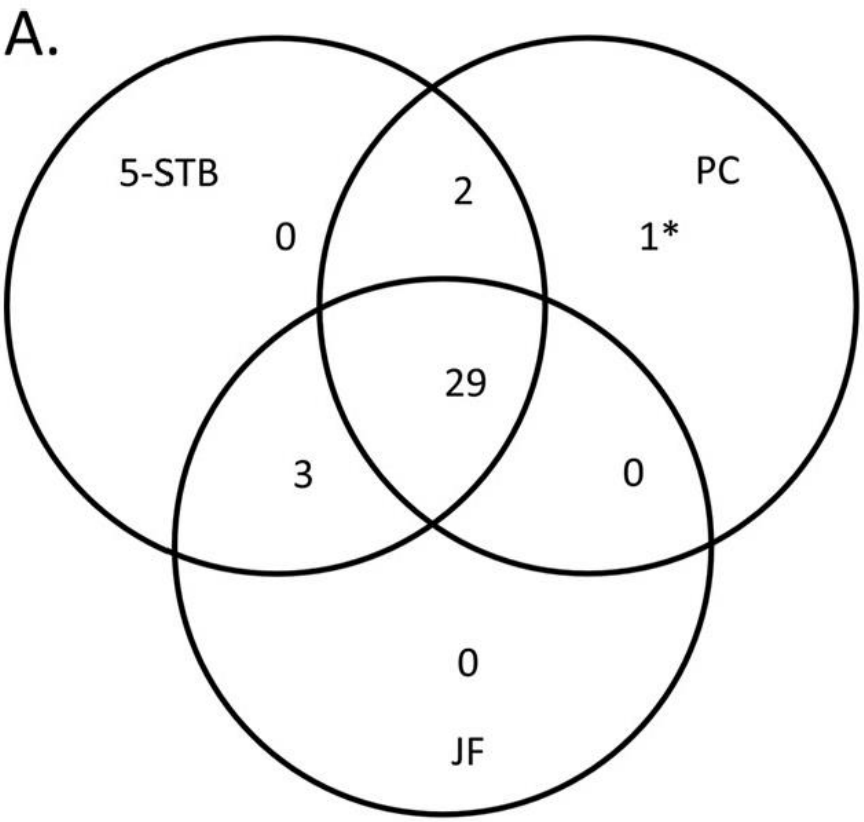
137 episodes (172 samples): PCR and culture from explanted valve material

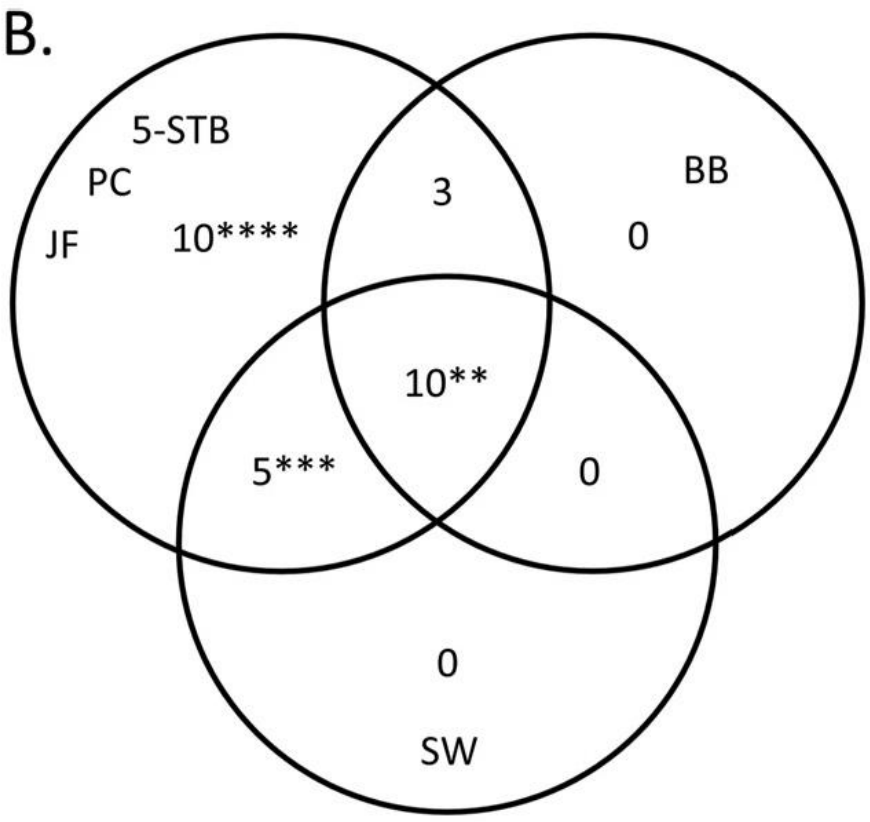
	PCR positive	PCR negative
Culture positive	22	77
Culture negative	38	

n=15: pathogen recovery from blood cultures
n=23: culture-negative

Christner, Rohde, in preparation

Diagnostic specimens: importance of tissue cultures





5-STB: 5 soft tissue biopsies
PC: prosthetic components
JF: Joint fluid

BB: bone biopsies
SW: prosthetic swab

Larsen et al., JCM 2018