Molecular investigation of bacterial communities on intravascular catheters: not just *Staphylococcus* anymore

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Running title: Bacterial communities on intravascular catheters.

Abstract

Intravascular catheter-related bloodstream infections (IVC-BSIs) are associated with significant morbidity and mortality. Culture-independent molecular approaches can reveal and capture the composition of complex microbial communities, and are now being used to reveal “new” pathogens as well as the polymicrobial nature of some infections. Patients with concurrently sited arterial and central venous catheters whom had clinically suspected IVC-BSIs, were examined by high-throughput sequencing of microbial 16S rRNA. An average of 100 operational taxonomic units (OTUs, phylotypes) was observed on each IVC, indicating that IVCs were colonized by complex and diverse bacterial communities. *Ralstonia* (53% of 16S rRNA sequences), *Escherichia* group (16%), *Propionibacterium* (5%), *Staphylococcus* (5%), and *Streptococcus* (2%) were the most abundant genera. There was no statistically significant difference in the bacterial communities examined from arterial and central venous catheters; from those with and without systemic antibiotic treatment; or from conventionally colonised and uncolonised IVCs. The genome of the predominant bacteria, *R. pickettii* AU12-08, was found to encode resistance to antimicrobial drugs of different classes. In addition, many encoded gene products are involved in quorum sensing and biofilm formation that would further contribute to increased antimicrobial drug resistance. Our results highlight the complex diversity of microbial ecosystems on vascular devices. High-throughput sequencing of 16S rRNA offers an insight into the pathogenesis of IVC-related infections, and opens up scope to improve diagnosis and patient management.

Keywords: intravascular catheter; catheter related bloodstream infections; biofilm; antibiotic resistance; high-throughput sequencing.

1 **Introduction**

2 Intravascular catheters (IVCs), including central venous catheters (CVCs) and arterial
3 catheters (ACs), are the most frequently used invasive medical devices in hospitals [1].
4 However, IVCs are associated with life threatening bloodstream infections (IVC-BSIs) which
5 have high associated rates of morbidity, mortality and additional personal and medical costs
6 [2,3].

7 Bacteria cause IVC-related infections when they gain access to the surface of IVCs and
8 establish biofilm, allowing sustained surface colonization and ultimately, dissemination into
9 the bloodstream leading to IVC-BSI. Early detection and adequate treatment of causative
10 pathogens is critical for a favourable outcome, yet the majority of patients with a suspected
11 catheter-related infection yield negative diagnostic investigations, necessitating empiric,
12 rather than optimal, antimicrobial therapy [4].

13 Unfortunately, less than 1% of bacteria in nature can be recovered using culture-based
14 methods [5]. Our previous studies have shown that many fastidious bacteria on medical
15 devices, potentially responsible for sepsis, are not diagnosed using current standard culture
16 examination as used in hospital laboratories [6]. Thus the most frequently isolated bacteria
17 might not be the dominant bacteria on colonised IVCs, or, indeed be responsible for many
18 patients' clinical conditions. The next generation sequencing now provides a key approach by
19 which to examine these complex microbial communities in a high-throughput manner.
20 Importantly, these approaches can reveal, and capture, the genetic potential present in
21 complex microbial communities without having to isolate and culture the microorganisms.
22 The cultivation-independent molecular approaches are now being used to reveal “new”
23 pathogens, as well as the polymicrobial nature of some infections [7,8].

24 Current strategies for control of IVC-BSI have focused more heavily on CVCs than ACs [9-
25 11]. However, the rate of IVC-BSIs arising from ACs has recently been demonstrated to be

1 comparable to that of CVCs [12-14], thereby rendering ACs an important device to study.
2 Little is known as regards to the microbial colonisation of CVCs and ACs sited concurrently
3 in the same patient as is a common situation in the intensive care unit (ICU) patient where
4 most patients need both IVCs. Despite the differing circulations and use of the two device
5 types, it is possible that bacteria which colonize one device may also colonize other
6 concurrently sited IVCs, in similar fashion, since the skin is the common portal of entry.
7 Because CVCs have been considered a higher infective risk than ACs, for patients with
8 suspected IVC-BSI, clinicians may remove the CVC but not the AC. Even if both catheters
9 are removed, it may be that the CVC is only sent for investigation, despite the AC
10 contributing equally to the infection, and being important for diagnosis and treatment. The
11 main aim of this study was to test this hypothesis and evaluate the bacterial profiles on the
12 surfaces of both ACs and CVCs removed concurrently from patients suspected of IVC-BSIs.

13 **Materials and Methods**

14 **Hospital setting and study population**

15 The study was carried out in the ICU of the Royal Brisbane and Women's Hospital (RBWH),
16 Queensland, Australia. This is a multi-disciplinary adult ICU that treats all conditions except
17 cardiac surgical and solid organ transplant. Fifteen adult patients (18 years of age or older)
18 with concurrently sited ACs and CVCs and with clinically suspected IVC-BSI were recruited
19 for the study (Table 1). Ethical approval for the study was granted by the RBWH Human
20 Ethics Board and Griffith University Human Research Ethics Committee. Written informed
21 consent was provided by participants or their representatives.

22 IVCs were inserted after skin decontamination with chlorhexidine in alcohol, by experienced
23 ICU medical staff using a Seldinger approach according to accepted guidelines for the
24 prevention of IVC-BSI [11]. All CVCs were ARROWgard Blue® (chlorhexidine acetate and

1 silver sulfadiazine coated) (Arrow Int, Inc, Reading, PA, USA), and ACs were Vygon Leader
2 Cath brand (Ecouen, France). There was no imposed limitation on dwell time, and resite of
3 catheters always occurred at a new body site. Guide-wire exchange was not performed.
4 Dressings and administration sets were maintained by ICU nurses using unit protocols in
5 accordance with guidelines [11]. If the attending intensive care specialist strongly suspected
6 that the IVC (either AC or CVC) could have been the cause of patient's signs and symptoms,
7 then both IVCs were removed and either replaced at a new site, or an alternative IV access
8 (e.g. peripheral vein) was sought. Diagnosis of IVC-BSI was made using conventional
9 methods [11].

10 IVC samples were cultured using the roll plate technique, and an IVC sample was considered
11 colonised if >15 cfu (colony forming unit) were isolated [15]. Microorganisms were then
12 isolated and identified according to standard hospital protocol. Data collected included
13 APACHE II score for severity of illness, patient demographics, antimicrobial use, catheter
14 dwell time and reason for removal, ICU and hospital length of stay.

15 **High-throughput sequencing of 16S rRNA**

16 Following processing for culture, catheter tips were suspended in 200 µl of lysis buffer,
17 which contained 20 mg/ml lysozyme, 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1.2% Triton,
18 and Proteinase K at 37 °C overnight. Bacterial genomic DNA was extracted from all IVCs
19 using the QIAamp DNA mini kit (Qiagen, Australia). For each catheter, a control (unused)
20 catheter was taken from the original packaging and rolled back and forth on blood agar
21 plates, with bacterial DNA extracted as above. 16S rRNA genes were amplified from purified
22 genomic DNA using the primers F (5' AGA GTT TGA TCC TGG CTC AG 3') and R (5'
23 CTG CTG CCT CCC GTA G 3') which would cover two variable regions (V1 and V2).
24 Thirty different self correction barcodes were designed and added to primers. For each DNA
25 sample, three replicate PCRs were performed. PCR products were cleaned through Qiaquick

1 PCR Purification kit (Qiagen, Australia). These were sequenced unidirectionally in the
2 reverse direction by means of the Genome Sequencer FLX (GS-FLX) system (Roche,
3 Australia) at 454 Life Sciences.

4 **Bioinformatics**

5 Raw 16S rRNA sequence reads were trimmed as follows. The base quality of reads tends to
6 decrease towards the 3'-prime end. To remove low quality 3'-ends, reads with an average
7 base quality below 25 were trimmed from the 3'-prime end until their average quality score
8 was above 25. The base quality of reads also considerably decreases after the first ambiguous
9 character ('N') (data not shown). Therefore, reads containing ambiguous characters were
10 additionally trimmed at the occurrence of the first 'N'. Subsequently, low quality reads were
11 identified and excluded using criteria adapted from Huse *et al.* 2007 [16].

12 All reads were assigned to Bergey's bacterial taxonomy using the RDP classifier and
13 iteratively grouped into Operational Taxonomic Units (OTUs) based on their best BLAST-hit
14 to full-length reference sequences from the RDP database. All reads with a best-BLAST hit
15 with a sequence-identity > 98%, were assigned to OTUs. Subsequently, all remaining reads
16 with a best-BLAST hit, with a sequence-identity > 95%, were then assigned to OTUs. The
17 remaining reads were assigned to OTUs analogously using identity cut-offs of 90% and 80%.
18 The Chao1 microbial richness estimate and overall community diversity (Shannon-Weaver
19 index) were computed from the OTU data. Rarefaction curves were generated by plotting the
20 number of observed genera versus the number of sequences sampled. Coordinates analysis
21 (PCoA) was carried out in R based on theta-similarities of the relative abundance of the
22 different genera in each sample. Sequences were chimera checked using ChimeraSlayer.
23 Anosim, Adonis and PERMDISP2 were calculated in Calypso. Anosim was run with Jaccard
24 distance as dissimilarity measure and patient as grouping. PERMDISP2 and Adonis were run

1 for the OTU relative abundance matrix with patients as grouping. The two tailed *t*-Test was
2 used to evaluate the difference between variances.

3 **Genome sequencing of *Ralstonia pickettii* AU12-08**

4 To maximize the recovery of bacteria, small pieces of IVC samples were cut and washed by
5 PBS and incubated in medium M₁₀ for 24 hours [17]. The solutions were then diluted in a
6 series (neat, 1:10, 1:50, 1:100) on Mueller-Hinton plates. As many different colony types as
7 visually distinguishable, were picked up, purified, and stored in glycerol at -80°C. Bacterial
8 DNA was released from bacterial cells by boiling, and one microliter was used as a template
9 in PCR amplification. Purified PCR products were then sequenced, and compared to NCBI
10 GenBank database using BLAST program. The most predominant bacterial species identified
11 in pyrosequencing were chosen for further study.

12 Bacterial metagenomic DNA from *Ralstonia pickettii* AU12-08 was extracted and genome
13 sequence of bacterial species was determined on the Genome Sequencer FLX (GS-FLX)
14 system (Roche, Australia) at 454 Life Sciences. Metagenomic contigs were *de novo*
15 assembled using GS De Novo assembler (version 2.3; Roche). Automatic genome annotation
16 was performed on the RAST server [18] and IMG/M-ER [19].

17 **Biofilm growth essay**

18 The assay to grow and quantitate biofilms has been used previously for other bacterial species
19 [20,21]. An overnight broth culture was diluted 1:200 in fresh broth and 200 µl was
20 inoculated into the wells of a 96 well Linbro tissue culture plate. The plates were incubated at
21 37°C, under 5% CO₂, for 12 days. Growth was then assessed by measuring the optical density
22 at 490 nm (OD₄₉₀) in a BioRad plate reader. To quantitate biofilm formation, 20 µl of Difco
23 crystal violet (Becton Dickinson, Australia) was added to each well and incubated at room
24 temperature for 15 minutes. Wells were washed vigorously with distilled water and the plate

1 was air dried. A volume of 230 µl of 95% ethanol was added to each well and the OD₅₇₀ was
2 measured. All strains were tested in quadruplicate. Each plate included 4 wells, which
3 contained sterile broth instead of bacteria, but were treated identically otherwise. The OD₅₇₀
4 was standardized against these wells.

5 **Accession number**

6 The draft genome of *Ralstonia rickettii* AU12-08 has been deposited at GenBank under
7 accession number ASZV 000000000.

8 **Results**

9 **Sample characteristics**

10 The 15 recruited patients had a mean age of 51.3 years, an APACHE II score of 23.6, ICU
11 stay of 16.7 days, and 80% were on systemic antimicrobials at the time of catheter removal
12 (Table 1). The mean duration of catheter placement was 5.6 days, and there was no difference
13 in dwell time between ACs and CVCs ($p>0.1$, two tailed *t*-Test).

14 According to the results of semi-quantitative cultures, 4 of the 30 removed IVCs were
15 considered colonised, and in these cases both the AC and CVC were colonised from two
16 patients. Isolates are including *Staphylococcus epidermidis* (catheter numbers 2A and 2C),
17 *Staphylococcus hominis* (3A), and mixed enteric and skin bacteria (3C) (Table 1). One of the
18 15 (6.66%) patients was diagnosed as IVC-BSI (matched tip and blood cultures with no other
19 explanatory source for the symptoms). Both colonisation (semi-quantitative method) and
20 IVC-BSI occurred in patients already receiving multiple antibiotics (see Table 1).

21 **Bacterial community profiles of IVCs**

22 No bacterial DNA was detected or amplified from negative control IVCs. These control
23 results indicate that the contribution to the bacterial community from manufacture of IVCs,
24 DNA extraction procedures and PCR reagents was negligible. Bacterial DNA was extracted

1 from each IVC sample and individually amplified. A total of 50,364 sequences (18.8%) did
2 not meet the quality control criteria and, thus, were excluded, and 217,488 high-quality
3 sequence reads were used for further analysis. Chimera checks showed that sequences were
4 not chimeric. Overall, microbial communities on both types of IVCs had a surprisingly high
5 diversity and complex community structure. 16S rRNA gene sequences were assigned to
6 sixteen bacterial phyla: Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria,
7 Deinococcus-Thermus, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospirae, OD1,
8 Proteobacteria, Spirochaetes, Tenericutes, Thermotogae, TM7 and unclassified bacteria (Fig.
9 1). The most dominant phylum was Proteobacteria (79.8% of 16S rRNA reads), which
10 included the taxonomic genus *Ralstonia* (53%), *Escherichia* group (16%), and
11 *Bradyrhizobium* (2%). The term of *Escherichia* group used in this study since the 16S rRNA
12 has difficulty on differentiating *Escherichia* and *Shigella*, and it was also suggested that
13 *Escherichia* and *Shigella* were sufficiently similar for placement in a single genus [22]. A
14 high number of OTUs was observed for each sample, ranging from 57 to 168 (Fig. 2). The
15 number of different genera per sample (bacterial richness) ranged from 22 to 59, and the
16 overall diversity (Shannon-Weaver index) was in the range of 3.09 to 4.08.

17 Microbial communities on ACs and CVCs had a similar community composition and
18 diversity. *Ralstonia* were dominant and accounted for 52.1% of examined sequences in ACs,
19 followed by *Escherichia* group (14.5%), *Staphylococcus* (5.2%), and *Propionibacterium*
20 (5.3%). CVCs had a similar community structure at an OTU level. Over 90% of identified
21 OTUs were present on both ACs and CVCs. Further, in terms of overall microbial diversity,
22 no difference was found between AC and CVC samples — the Shannon-Weaver diversity
23 index values for both ACs and CVCs were around 3.5 ($p>0.05$, two tailed t -Test). All results
24 indicated no statistically significant differences in the bacterial communities examined from
25 CVCs and ACs ($p>0.05$, two tailed t -Test). The PCoA (Fig. 3a) suggested that bacterial

1 communities on ACs and CVCs had similar community profiles, as the AC and CVC
2 microbial communities did not form distinct clusters. Thus, incidence of bacterial
3 colonisation and, therefore, the potential for causing IVC-BSI did not appear to differ
4 between ACs and CVCs. These findings support the assertion that ACs should be considered
5 as an equally likely site, as CVCs, of catheter colonization in critically ill patients [12].

6 For the majority of patients (11/15) the microbial communities found on the two different
7 catheters were more similar than the average distance of microbial communities from
8 different patients. The average Jaccard distance of communities from different patients was
9 0.6, the average Jaccard distance of communities from the same patient was 0.39 ($p=0.12$, t-
10 test) (Fig. 4). According to Anosim, Adonis and PERMDISP2, the intra-patient communities
11 were generally more similar than between-patient communities (Anosim: $p=0.002$; Adonis:
12 $p=0.001$; PERMDISP2: $1e-225$).

13 We compared the bacterial communities present on ‘colonised’ and ‘uncolonised’ IVCs. The
14 dominant bacteria on both groups were *Ralstonia* and *Escherichia* group. No statistically
15 significant differences were found between these two groups in terms of bacterial profiles,
16 OTU distribution, Shannon-Weaver diversity, and PCoA (Fig. 3b). Moreover, it was also
17 noticed that for patient number 2, who had both IVCs ‘colonised’ by coagulase-negative
18 *staphylococcus* (CNS) and *Staphylococcus* spp. using the roll-plate culture method,
19 *Staphylococcus* spp. in fact represented only 3% of the bacterial community present on both
20 IVCs via molecular methods. Further, for patient number 15, the AC and CVC yielded sub-
21 diagnostic growth (<15 cfu) of *Staphylococcus* spp., and for mixed *Staphylococcus* and
22 *Escherichia* group respectively, whereas over 60% of 16S rRNA sequences from the AC
23 were *Staphylococcus* spp. and 72% of 16S rRNA gene sequences from CVC were
24 *Acinetobacter* spp. Bacterial communities on this patient’s IVCs were different from the

1 remaining patients' IVCs, which were dominated by species closely related to *Ralstonia*
2 and/or *Escherichia* group.

3 Eight IVC samples (4 ACs and 4 CVCs) came from four patients not receiving antibiotic
4 treatment. We compared the bacterial communities on these samples to the 22 IVC samples
5 from 11 patients treated with intravenous antibiotics in the two weeks prior to sample
6 collection. Statistically, there was no significant difference in the bacterial communities on
7 IVCs from patients with, or without, antibiotic treatment, as confirmed by the results of
8 PCoA (Fig. 3c) and OTU distribution.

9 ***Ralstonia pickettii* AU12-08 genome sequencing**

10 The closest cultured relative of AU12-08 is *R. pickettii*, and it shares 100% sequence identity
11 with the 16S rRNA genes of the predominant bacterial species in pyrosequencing libraries.
12 Therefore, a thorough understanding of *R. pickettii* AU12-08 genome is vital for the
13 systematic understanding of bacterial pathogenesis on IVC-related infections. The sequence
14 data consists of 6,229,152 bp chromosome with G+C content of 63.6%. The *R. pickettii*
15 AU12-08 genome harbours 50 tRNA genes coding for all amino acids, and 5733 predicted
16 protein coding genes consistent with other sequenced *Ralstonia* spp. [23,24]. Comparisons of
17 the general genomic features of *R. pickettii* AU12-08 with nosocomial pathogenic *R. pickettii*
18 strains 12J and 12D, and environmental isolate *R. solanacearum* GM1000, are shown in
19 Table 2.

20 A seven gene operon coding for *Pel* synthesis machinery was present in *R. pickettii* AU12-08.
21 *Pel* was originally found to be responsible for the production of glucose-rich biofilm matrix
22 exopolysaccharide in strain *Pseudomonas aeruginosa* 14 [25]. The detailed comparison of
23 *Pel* coded by *R. pickettii* AU12-08 genome with *R. pickettii* 12J, *R. pickettii* 12 D and PA 14,
24 showed a high degree of similarity with respect to gene organization and predicted function

(Fig. 1Sa). An independent study by Vasseur et al. showed that glucose-rich polysaccharides are essential for the formation of a surface-associated biofilm; thus, it is a major component of biofilm in strain *Pseudomonas aeruginosa* K [26]. The *pel* operon contains seven genes, *pelA* to *pelG*, which display sequence similarity with genes that encode sugar-processing enzymes. These include oligogalacturonide lyase (*pelA*), glycosyltransferases (*pelC* and *pelF*), sucrose synthase (*pelE*), and transmembrane proteins (*pelD* and *pelG*). Transposon insertion into, or deletion of, these genes resulted in severe defects in biofilm formation [26].

More genes in the *R. pickettii* AU12-08 genome were found to encode resistance to antibiotics and toxic compounds compared to *R. pickettii* 12J, *R. pickettii* 12D and *R. solanacearum* GM1000 (table 2). The *R. pickettii* AU12-08 genome contains 22 putative Multidrug Resistance Efflux Pumps (resistance nodulation division family), which enable bacteria to excrete antibiotics and other toxic compounds. Multidrug Resistance Efflux Pumps have been previously demonstrated to not only confer resistance to drugs used in therapy, but also to have a role in bacterial pathogenicity, i.e., through bacterial colonization and bacterial survival in the host [27]. Seven genes in chromosome 1, and 3 genes in chromosome 2 of *R. pickettii* AU12-08 genome, were coding for beta-lactamase including beta-lactamase (EC 3.5.2.6), beta-lactamase class C, and penicillin binding proteins and beta-lactamase class D. Environmental isolate *R. solanacearum* GM1000 does not encode beta-lactamase (EC 3.5.2.6), beta-lactamase class C or penicillin binding proteins. *R. pickettii* AU12-08 shares a high similarity with PA14 on beta-lactamase which has been reported to resist Beta-lactam antibiotics (Fig. 1Sb). In addition, the presentation of *gyrA*, *gyrB*, *parC* and *parE* in *R. pickettii* AU12-08 might indicate the resistance of fluoroquinolones. Thirteen genes were coded for components of tripartite multidrug resistance system. Further, 170 genes were coded for resistance toxic compounds, including cobalt-zinc-cadmium, copper homeostasis, mercury operon, arsenic and bile hydrolysis.

1 **Biofilm essay**

2 *R. pickettii* AU12-08 recovered from IVC tips was studied for biofilm formation. To
3 characterise the kinetics of biofilm formation, the levels of biofilm formation were measured
4 after 3, 6, 9 and 12 days respectively. We also examined the effect of other bacterial species
5 (*E. coli* and *S. aureus*) on the general growth characteristics of *R. pickettii* AU12-08 (Fig. 5a).
6 We compared the biofilm formation of *R. pickettii* AU12-08 to that of other bacterial species
7 by measuring the OD₅₇₀. As shown in Fig. 4, throughout duration of the biofilm formation,
8 the biofilm formed by *R. pickettii* AU12-08 alone, was less than that when formed in
9 combination with multiple bacterial species (Fig. 5b). These results suggest that *R. pickettii*
10 AU12-08 forms biofilms independently, and also in a greater volume of biofilms when
11 present with other bacterial species.

12 **Discussion**

13 Using high-throughput 16S rRNA sequencing, we demonstrated the complex diversity of the
14 microbiological flora on commonly used intravascular catheters. An understanding of the
15 composition of bacterial communities on IVCs is essential for the prevention and treatment of
16 IVC-BSI. Many identified bacterial species in this study were known pathogens or
17 opportunistic pathogens such as *Ralstonia* spp., *Escherichia* group spp., *Staphylococcus* spp.,
18 *Streptococcus* spp., *Acinetobacter* spp., *Chryseobacterium meningosepticum*,
19 *Corynebacterium* spp., *Stenotrophomonas* spp. and *Pseudomonas* spp.

20 The predominant bacterial species on examined IVCs were closely related to *R. pickettii*
21 (over 50% of examined sequences). *R. pickettii* (*Pseudomonas pickettii*) is a non-
22 fermentative, gram-negative bacterium isolated from environmental and clinical samples. *R.*
23 *pickettii* has the ability to survive in 0.05% chlorhexidine solutions [28] and penetrate 0.2 mm
24 filters [29]. Therefore, many of the cases of infection with *R. pickettii* were due to
25 contaminated water or aqueous solutions given intravenously [30]. Even “sterile” medical

1 products including ranitidine and saline solution had been reported to be contaminated with
2 *R. pickettii* [31,32]. Several nosocomial catheter-related outbreaks have been described
3 [30,33,34].

4 *R. pickettii* growth is slow and unfavourable on the blood agar plates currently used for
5 routine IVC culture, which may explain why clinical results are generally negative for this
6 organism. Our results challenge the belief that *R. pickettii* is of low virulence and incidence in
7 IVCs, and suggest that it is, in fact, embedded in a biofilm and thus escaping detection using
8 current methods. It may be of virulence itself, or it may be important in the subsequent
9 colonisation or activity of other microbes. *R. pickettii* AU12-08 contains genes important for
10 biofilm formation. A seven gene operon coded for *Pel* synthesis machinery. *Pel* was
11 originally found to produce glucose-rich biofilm matrix exopolysaccharide in *Pseudomonas*
12 *aeruginosa* 14. The biofilm assay used in this study demonstrated that *R. pickettii* AU12-08 is
13 capability of forming biofilms.

14 Patients recruited within this study were treated with vancomycin, meropenem and
15 piperacillin/tazobactam (Table 1). Vancomycin is used to treat infections caused by gram-
16 positive bacteria, meropenem is a beta-lactam and piperacillin/tazobactam combines the
17 extended-spectrum penicillin piperacillin and β -lactamase inhibitor tazobactam. The genome
18 of *R. pickettii* AU12-08 harboured numerous genes coding for resistance of antibiotics and
19 toxic compounds. *R. pickettii* AU12-08 has 22 putative multidrug resistance efflux pumps to
20 enable bacteria to excrete antibiotics and toxic compounds in catheter coatings. *R. pickettii*
21 AU12-08 are gram-negative bacteria, therefore they are not sensitive to vancomycin. *R.*
22 *pickettii* AU12-08 also contains β -lactamase, which can break the β -lactam (meropenem)
23 structure. *R. pickettii* AU12-08 possesses genes encoding proteins involved in biofilm
24 formation, that also contribute to increased antimicrobial drug resistance. Considering that
25 most patients were receiving antibiotic treatment, yet most IVCs had *R. pickettii* present, it

1 would appear that it is able to resist current therapy. Whilst the origin of the detected bacteria
2 cannot be ascertained from our study, it is possible that some organisms may have been
3 environmentally acquired. Since all controls were negative, it is unlikely that contamination
4 occurred in the laboratory. Our study did not include specimens from patients who were not
5 suspected of infection at the time of catheter removal, and this could be a focus of future
6 studies, as long as the dwell time of catheters was matched between groups. Colonization of
7 the indwelling IVC can occur via health care workers hands if they contaminate the hub or
8 infusates while giving medications and fluids, or when drawing blood. This is plausible since
9 these IVCs are typically accessed for therapy many times per day - staff hand hygiene and
10 administration set decontamination prior to access may not always be optimal. Other
11 organisms we identified may be resident on patients' skin – gram positives but also gram-
12 negative bacteria, which increase in density on the skin and heightened the increased acuity
13 of illness and duration of hospitalization typical in ICU patients. To verify with certainty the
14 source of these bacteria would require a comprehensive assessment beyond the scope of the
15 current study.

16 **Conclusions**

17 This study demonstrates the utility of high-throughput sequencing for examining bacterial
18 communities on medical devices. Many microorganisms that were encountered were closely
19 related to known human opportunistic or true pathogens and some have been commonly
20 involved in IVC-related infections. Description of these bacterial communities will improve
21 our understanding of IVC-related infections since bacterial colonization is the first step
22 leading to IVC-related infections. In addition, better understanding of these bacterial
23 communities may aid the development of more efficient diagnostic and therapeutic
24 approaches for IVC-related infections, both of which remain difficult in modern medicine.

25

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2 **Acknowledgments**

3 We thank Jenny Murfield for editorial assistance with the manuscript. Dr. Li Zhang is
4 supported by Australian National Health and Medical Research Council Training Clinical
5 Research Fellowship, Australian Government (grant number 597491). The research was
6 supported by project grants from Griffith Health Institute and Health Practice Innovation
7 (Griffith University, Australia.

8 **Conflict of Interest**

9 All authors declare no conflicts of interest relevant to this article.

10

1

2 **Table 1. Patient demographics and roll-plate intravascular catheters culture results.**

Patient number	ICU stay (days)	Diagnosis	APACHE II score	Antibiotic usage	'Roll-plate' culture result
1	14	Subdural hematoma	27	Meropenem and Vancomycin	NG
2	17	Sepsis pneumonia	27	Vancomycin, Piperacillin and Tazobactam	2A: 100 cfu <i>Staphylococcus epidermidis</i> 2C: 10 ³ cfu mixed CNS
3	12	Peritonitis	22	Piperacillin and Tazobactam	3A: 50-100 cfu <i>Staphylococcus hominis</i> 3C: 50-100 cfu mixed <i>Staphylococcus</i> and <i>Escherichia</i>
4	8	Cardiac arrest	29	None	NG
5	7	Sepsis	18	None	NG
6	9	Respiratory failure	19	None	NG
7	14	Subdural hematoma	27	None	NG
8	38	Respiratory failure	30	Piperacillin and Tazobactam	NG
9	10	Burn	16	Piperacillin and Tazobactam	NG
10	8	Fecal peritonitis	13	Meropenem and Vancomycin,	NG
11	32	Subarachnoid hemorrhage	19	Vancomycin, Meropenem, Piperacillin and Tazobactam,	NG
12	12	Craniotomy	30	Meropenem, Piperacillin and Tazobactam	NG
13	38	Multi-trauma	21	Piperacillin and Tazobactam	NG
14	19	Cardiac arrest	36	Piperacillin and Tazobactam	NG
15	13	Liver failure	20	Meropenem, Vancomycin, Piperacillin and Tazobactam,	NG

3

4

5

6

1 **Table 2. General features of genomes of *R. pickettii* strains AU12-08, 12J, 12D and *R.***
2 ***solanacearum* GM1000.**

	<i>R. pickettii</i> AU12-08	<i>R. pickettii</i> 12J	<i>R. pickettii</i> 12D	<i>R. solanacearum</i> GM1000
Origin	Clinical isolate	Nosocomial pathogen	Nosocomial pathogen	Environmental isolate
Length (Mb)	6.23	5.33	5.69	5.81
GC%	63.7	63.6	63.3	67
#CDs	5733	5100	5361	5635
tRNA	50	57	54	57
Virulence factors				
Multidrug resistance efflux pumps	22	18	18	13
Beta-lactamase	10	4	4	3
Multidrug resistance tripartite systems	13	3	3	11
Resistance to fluoroguinolones	4	4	5	4
Resistance to toxic compounds	168	128	112	55
Quorum sensing and biofilm formation	9	12	13	12

3

1 **Figure Legends**

2 **Fig. 1. Microbial genera detected in intravascular catheters.** A represent arterial catheters
3 and C represent central venous catheters.

4 **Fig. 2. Heat map depicting relative abundance of bacterial genera across intravascular**
5 **catheters samples of 15 patients.** A represent arterial catheters and C represent
6 central venous catheters.

7 **Fig. 3. Principal coordinate analysis of bacterial communities of intravascular catheters**
8 **samples based on UniFrac distances.** (a) 15 samples; (b) colonised IVCs (grey balls)
9 and uncolonised IVCs (black balls); (c) antibiotic treatment (red balls) and without
10 antibiotic treatment (blue balls). Each point corresponds to each IVC sample.

11 **Fig. 4. Jaccard distance measuring dissimilarity between and intra 15 patients.**

12 **Fig. 5. (a) Time course of biofilm formation of five groups of bacteria. (b) Biofilm**
13 **formation of five groups of bacteria.** The data represent the average absorbance at
14 570 nm ($\pm$ s.e.m.). A, *Ralstonia pickettii*; B, *Pseudomonas aeruginosa*; C, *Escherichia*
15 *coli*; D, *Staphylococcus aureus*; E, *Ralstonia pickettii* together with *Escherichia coli*
16 and *Staphylococcus aureus*.

17 **Fig. 1S. (a) Comparison of extracellular matrix proteins (*Pel*) maps reconstructed from**
18 ***R. pickettii* AU 12-08 and other five bacterial strains.** 1, *Pel* B; 2, *Pel* C; 3, *Pel* A;
19 4, *Pel* D; 5, *Pel* E (987 bp); 6, *Pel* F; 7, *Pel* G. (b) **Beta-lactamase from *R. pickettii***
20 **AU 12-08. (c) Multiple drug efflux pumps from *R. pickettii* AU 12-08.**

21

1 **Fig. 1.**

2

3

4

1 **Fig. 2.**

2

3

1 **Fig. 3.**

2 **(a)**

3

4 **(b)**

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6

1 (c)

2

3

1 **Fig. 4.**

2

3

1 **Fig. 5.**

2 **(a)**

3

4 **(b)**

5

6

7

1 **Fig. 1S.**

2 **(a)**

3

4

1 (b)

2

3 BLC represents beta-lactamase class C and penicillin binding protein; BLD represents beta-lactamase class D.

4

5

1 (c)

2

3

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