

**The potential of different molecular biology methods in tracking clones of
Acinetobacter baumannii in an ICU setting.**

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Running Title: Molecular methods in tracking clones of *A. baumannii*

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24 **Abstract (248 words)**

25 **Purpose:** This study aimed at characterising *A. baumannii* strains isolated from patients in
26 ICU setting using molecular techniques to study clonal relatedness to determine a fast,
27 efficient and a cost effective way of detecting persistent clones.

28 **Methodology:** *A. baumannii* (n=17) were obtained in June and November 2015 from a single
29 ICU setting in South India. DNA typing methods such as MLST, SBT and DNA fingerprinting
30 PCRs (M13, DAF4, ERIC2) were employed to understand the association of clones. PCR for
31 antimicrobial resistance genes *ISAbal-bla*_{OXA-51-like}, *ISAbal-bla*_{OXA-23-like}, *bla*_{NDM-1}, *bla*_{PER-7},
32 *bla*_{TEM-1} and virulence genes *cpa 1*, *cpa 2* and *pkf* were performed.

33 **Results:** The MLST showed some degree of corroboration with other DNA typing methods
34 such as SBT and M13, DAF4, ERIC2. M13 PCR was found to give better results than other
35 fingerprinting methods. ST848 (CC92) was the dominant strain isolated in both June and
36 November. All isolates were *bla*_{OXA-51-like} positive with 16 having *ISAbal* upstream of *bla*_{OXA-}
37 51-like and *bla*_{OXA-23-like} genes. Genes such as *bla*_{NDM-1} (23%, n= 4), *bla*_{PER-7} (58.8%, n= 10), *pkf*
38 (82%, n= 14), *bla*_{TEM-1} (5.8%, n=1), *cpa 1* (5.8%, n=1) and *cpa 2* (5.8%, n=1) were also
39 detected.

40 **Conclusion:** M13 PCR can be used in routine environmental surveillance for detection of
41 persistent antibiotic resistant clones in an ICU setting because of its reliability and simplicity.
42 Further studies based on greater sample size, conducted at multi-centre level can give a
43 better understanding on the reliability of molecular methods which can be used for
44 detection of persistent clones in hospital setting.

46 **Introduction:**

47 Nosocomial infections are a major problem for immunocompromised patients, increasing
48 mortality and morbidity rates, even in a specialized and well-equipped hospital
49 environment. The main cause of nosocomial infections is bacterial drug resistance to various
50 antimicrobial agents and the difficulty in eradicating them. *Acinetobacter baumannii* is an
51 opportunistic pathogen of clinical significance which causes various hospital and
52 community-acquired infections such as pneumonia, urinary tract infection, suppurative
53 infection and bloodstream infections [1]. It is not only emerging as a cause of numerous
54 global outbreaks but also has increasingly high rates of resistance which makes treatment
55 difficult for severely ill patients that need hospitalisation [2–4]. The frequency by which the
56 intubated patient acquires *A. baumannii* infection is around 58% compared to 30% which is
57 caused by other Gram-negative bacteria [5].

58 The most important mechanisms through which *A. baumannii* can acquire resistance are:
59 presence of intrinsic antimicrobial resistance (AMR) genes such as oxacillinases, insertion
60 sequences like *ISAbal* which help in mobilisation of such genes and control their [6, 7].
61 Virulence genes such as serine protease (*pkf*) which degrades the essential complement
62 components like C3 and endopeptidases (*cpa1* and *cpa2*) which disrupt the common
63 mechanism of coagulation, help the organism to prevail and disseminate in the hospital
64 setting [8, 9]. Resistance Nodulation Division (RND) family efflux pumps genes such as
65 *adeABC*, *adeIJK* and *adeFGH* also play an important role in the emergence of resistant *A.*
66 *baumannii* strains [10].

67 The exact mode of transmission may not be known, because the isolation of *A. baumannii*
68 strains in environmental surveillance cultures is not so frequent which makes eradication of

this organism very difficult in the hospital setting but it can be prevented by implementing good clinical practises and effective infection control measures [11–14]. Most of the studies consistently report on the nosocomial infection caused by *A. baumannii*. The hospital outbreaks and nosocomial infection by *A. baumannii* has been attributed to their increase antibiotic resistance, virulence and their ability to survive on inanimate and dry surface [4, 5, 9, 10]. To understand more about the spread and epidemiology of *A. baumannii* in a hospital setting many molecular based methods have been employed. They can be broadly classified as 1) PCR-based DNA fingerprinting techniques (M13, ERIC, DAF4) 2) Restriction enzyme based (AFLP, RFLP and PFGE) 3) Sequence based (Single locus sequence based typing) 4) Single nucleotide polymorphism based on analyses of whole genome and 5) Multi locus sequence typing (MLST) [15]. The main aim of this study was to identify and track the circulating clonal types of *A. baumannii* in an Intensive care unit (ICU) setting to detect the mode of transmission between the patients in the months of June and November of 2015 using molecular techniques such as MLST, SBT and DNA fingerprinting PCRs. This project also compares the efficacy of the molecular techniques based on cost, time and its effective usage in routine surveillance to identify circulating clonal types in hospital setting.

Materials and Methods:

Bacterial isolates:

Acinetobacter baumannii isolates were obtained from a tertiary care hospital, in South India, in June, 2015 (AB01-AB12) and November, 2015 (AB13 to AB 17) (Table S1). All isolates were from the same ICU setting and were either multidrug resistant (resistant to more than three classes of antibiotics) or extensively drug resistant (susceptible to two or less than two classes of antibiotics)[16]. The isolates were from blood and endotracheal aspirate (ETA). In

case of ETA isolates there were 3 inclusion criteria: The patient sample should have more than 10^5 CFU/ml of organisms, the pus cells should be moderate or more and radiological features must suggest ventilator associated pneumonia. Isolates which fulfilled at least two criteria were selected (Table S1). All the isolates were identified as *Acinetobacter baumannii* using PCR amplification of *bla*_{OXA-51-like} gene and confirmed by sequencing of *rpoB* gene [17–19].

Antimicrobial Susceptibility Testing:

Susceptibility pattern to different classes of antimicrobials were determined by standard disc diffusion method. The results were interpreted according to Clinical Laboratory Standard Institute guidelines [20, 21]. The antimicrobials used for disc diffusion susceptibility testing were ceftazidime (30 µg), cefepime (30 µg), piperacillin/tazobactam (100/10 µg), cefoperazone/sulbactam (75/30 µg), amikacin (30 µg), netilmicin (30 µg), tobramycin (10 µg), aztreonam (30µg), levofloxacin (5 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), imipenem (10 µg) and meropenem (10 µg). Minimum inhibitory concentration (MIC) by broth micro dilution (BMD) for polymyxin B and polymyxin E were performed for all clinical isolates of *A. baumannii* as per the CLSI guidelines. *A. baumannii* ATCC 19606 was used as the control strain.

CarbAcineto NP:

CarbAcineto NP test was performed for the phenotypic assessment of carbapenemase activity for all *A. baumannii* strains [22]. The carbapenemase activity was detected by colour change from red to yellow, which results by the hydrolysis of imipenem leading to

decrease in pH value. Strains BAA-1705 (*bla*_{KPC} positive) and BAA-1706 (carbapenemase negative) were included as positive and negative controls.

PCR for the detection of carbapenemase genes:

DNA extraction was performed using automated Qiagen QiaSymphony system as per the manufacturer's instructions [23]. Multiplex PCR for the detection of OXA classes of enzymes was performed as described earlier by Woodford et al [24]. A multiplex PCR was also performed to detect extended spectrum β -lactamases (ESBL) genes such as *bla*_{TEM}, *bla*_{SHV}, *bla*_{GES}, *bla*_{PER}, *bla*_{VEB} and class A carbapenemase *bla*_{KPC} and for the detection of *bla*_{IMP}, *bla*_{VIM}, *bla*_{SIM}, *bla*_{NDM}, and *bla*_{SPM} metallo β -lactamases [25–28].

In order to detect allelic variants of genes, primers (Table S2) were designed using BioEdit (<http://www.mbio.ncsu.edu/BioEdit/page2.html>) and NCBI gene sequences. PCR was performed using Veriti thermal cycler, Applied Biosystem. The cycling conditions were: initial denaturation at 94 °C for 15 min, 35 cycles of 94 °C for 30 sec, 54 °C for 30 sec, 72 °C for 45 sec, followed by final extension at 72 °C for 10 min. The amplified products were sequenced using AB - Applied Biosystem 3130 - Genetic Analyser and then the sequence information was analysed and translated into amino acid sequences using BioEdit software. The translated information was used for blastp analysis using the Protein Basic Local Alignment Search Tool available through NCBI [29].

Detection of insertion element associated with OXA51 and OXA23:

To detect the presence of insertion sequence (IS) *ISAb_a1*, PCR was first performed using primers described by Turton et al [30]. Also, mapping of *ISAb_a1* element relative to *bla*_{OXA-23-like} and *bla*_{OXA-51-like} genes was accomplished. In case of *bla*_{OXA-23-like}, forward primer of *ISAb_a1*

with reverse of *bla*_{OXA-23-like} and reverse of *ISAb_a1* with forward primer of *bla*_{OXA-23-like} was used, while in *bla*_{OXA-51-like}, FxOxa forward primer described by Lopes et al. (2011) and reverse of *bla*_{OXA-51-like} were used (Table S2)[7, 24].

For mapping *bla*_{OXA-51-like}, Qiagen Phusion high fidelity PCR master mix from New England Biolabs was used. The reaction mix consisted of 2 µl of template DNA, 10 µl of Phusion master mix, 1 µl each of forward and reverse primer (10 pM), 0.6 µl DMSO and 5.4 µl of molecular grade water. The cyclic conditions for *bla*_{OXA-51-like} mapping were; initial denaturation at 95°C for 2 minutes, 35 cycles of 95°C for 10 secs, 57°C for 30 secs, 72°C for 30 secs, followed by final extension at 72°C for 10 minutes. For mapping of *ISAb_a1-bla*_{OXA-23-like} Qiagen HotStart PCR master mix was used. The reaction mix consisted of 2 µl of template DNA, 10 µl of HotStart master mix, 1 µl each of forward and reverse primer (10 pM) and 6 µl of molecular grade water. The cycling conditions were; initial denaturation at 95°C for 15 minutes, 35 cycles of 95°C for 30 secs, 56°C for 45 secs, 72°C for 1 minute followed by final extension at 72°C for 10 minutes.

PCR for detection of virulence genes:

PCR was performed to detect serine protease gene *pkf* and zinc-dependent metallo-endopeptidase genes *cpa1* and *cpa2* in this study (Table S2). The reaction mix consisted of 2 µl of template DNA, 10 µl of Qiagen HotStart master mix, 1 µl each of forward and reverse primer (10pM) and 6 µl of molecular grade water. The cycling conditions for *pkf*, *cpa1* and *cpa2* were as follows: initial denaturation at 95°C for 15 minutes, 35 cycles of 95°C for 45 secs, 52°C for 45 secs, 72°C for 2 minutes, followed by final extension at 72°C for 10 minutes.

Multilocus sequence typing:

All *A. baumannii* isolates were subjected to MLST analysis using the Oxford scheme with primers and conditions described previously by Bartual et al [31]. The amplicons were purified using Magbio kit (Gaithersburg, US) and sequenced using the AB - Applied Biosystem 3130- Genetic Analyser. The sequences obtained were trimmed using FinchTV and BioEdit. After trimming the sequence were submitted in PubMLST data base and Sequence types (STs) were assigned. New STs and allele numbers were assigned by submitting the data to the curator of *A. baumannii* in MLST database. The clonal complexes (CCs) were established using the eBURST software (<http://eburst.mlst.net/>)[32].

Single-locus sequence based typing:

Another technique used for distinguishing *A. baumannii* is single-locus sequence based typing of *bla*_{OXA-51-like} which is an intrinsic class D beta-lactamase gene in this species [33]. The *bla*_{OXA-51-like} variants were analysed using the Oxa 51F and Oxa 51R primers (Table S2). The cycling condition used were; Initial denaturation at 95°C for 15 minutes, 35 cycles of 95°C for 1min, 50°C for 2min, 72°C for 3min, followed by final extension of 72°C for 10 minutes. The amplicons were purified using Magbio kit and sequenced using the ABI-applied biosystem 3130- genetic analyser sequencing. The sequences obtained were trimmed and translated to their respective protein sequence using BioEdit. The translated sequence was blasted in NCBI using protein blast tool (blastp) to detect *bla*_{OXA-51-like} variants.

PCR based DNA fingerprinting:

DNA fingerprinting analyses were performed using ERIC2, 5'-AAGTAAGTGACTGGGGTGAGCG-3', M13, 5'-GAGGGTGGCGTTCT-3' and DAF4, 5'-

179 CGGCAGCGCC-3' primers as described earlier [34]. Primers M13 and DAF4 target conserved
180 sequences, while primer ERIC-2 targets enterobacterial repetitive sequences [34]. Slight
181 modifications were done in the cycling condition to obtain optimal product amplification,
182 initial denaturation was performed at 95°C for 15 mins, followed by 35 cycles of 95°C for 1
183 min, 50°C for 2 mins, 72°C for 3mins, and final extension of 72°C for 10 mins for all three
184 primer sets.

185

186 Cluster analysis was performed using the unweighted pair group method with mathematical
187 averages, and relatedness was calculated using the band-based Dice coefficient with a 1.5%
188 band tolerance and 1.5% optimisation setting for the whole profile using BIONUMERICS v7.6
189 software (Applied Maths, Sint-Martens-Latem, Belgium). A value of 85% was chosen as the
190 threshold for the establishment of clonal relatedness of the strains [35].

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Results:

Antimicrobial Susceptibility Testing:

All isolates except AB17 were resistant to 7 classes of antimicrobials including; amikacin, aztreonam, ceftazidime, ciprofloxacin, imipenem, levofloxacin, meropenem, netilmicin, piperacillin/tazobactam, sulbactam, tobramycin, trimethoprim/ sulfamethoxazole. For carbapenem resistance the AST data was well supported by CarbAcineto NP which gave positive result for 94% (n=16) of the isolates. This test had 100% sensitivity (95%CI: 79.41% to 100.00%) and 100% specificity (2.50% to 100.00%). In case of colistin all isolates were susceptible, whereas for tigecycline and tetracycline the resistance was seen in 70% (n = 12) and 35% (n = 6) of isolates.

Antimicrobial resistance genes:

The intrinsic oxacillinase gene *bla*_{OXA-51-like} was found in all 17 isolates of which 15 isolates were positive for *ISAbal-bla*_{OXA-23-like} constituting a *Tn2008* while one isolate had *ISAbal-bla*_{OXA-23-like-ISAbal} constituting a *Tn2006* transposon. The *bla*_{NDM-1} metallo β-lactamases was detected in 23.5% (n=4) isolates, other genes such as *bla*_{KPC}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{SIM}, *bla*_{SPM}, *bla*_{OXA-24-like} and *bla*_{OXA-58-like} could not be detected. 35.2% of the isolates (n=6) were negative for tested ESBL encoding gene while *bla*_{PER-7} was present in 58.8% of isolates (n=10) and *bla*_{TEM-1} gene in 1 isolate (Table 1). The *bla*_{SHV}, *bla*_{VEB} and *bla*_{GES} genes were not identified in any isolates. *ISAbal* element was present in all the isolates but in 94.1% of isolates (n=16), this element was found upstream of *bla*_{OXA-51-like} gene (Table 1). Among the 17 isolates tested, 14 isolates (82.3%) were positive for proteinase kinase F (*pkf*) and one isolate positive for both *cpa1* and *cpa2* coagulation dependent metallo-endopeptidase (Table 1).

Multilocus and Single-locus sequence based typing:

Among the STs identified by MLST (Oxford scheme), five were novel and assigned as ST1305, ST1306, ST1307, ST1308 and ST1335 and were identified in five isolates. Other known strains, ST1114, ST391 and ST218 were detected in three isolates. ST848 strain was detected in 5 isolates in both June and November months, followed by ST862 identified in 2 isolates and ST195 in 2 isolates. (Table 1). The eBURST analyses of our strains with all identified STs in MLST database showed that ST195, ST218, ST848, ST1114, ST1305 belonged to CC92, whereas ST391 belonged to CC391 and ST862, ST1306, ST1308 belonged to CC862, whereas ST1307 and ST1335 were singletons (Figure 1). SBT was carried out for all 17 isolates of which 11 isolates carried the *bla*_{OXA-66} variant, 3 isolates were *bla*_{OXA-104} positive, 3 isolates were positive for one of the OXA-variant, *bla*_{OXA-68}, *bla*_{OXA-144} or *bla*_{OXA-343}.

PCR based DNA fingerprinting:

Of the three different primer sets, M13 primer generated distinct fingerprint patterns, with only a relatively small number of secondary faint bands (Figure 2). All DNA fingerprinting methods generated at least 9 distinct clusters in which isolates could be grouped. Using the DAF4 primer seven isolates (cluster 1) grouped together of which five ST848 (CC92, OXA-66) isolates clustered together in the ERIC2 (cluster 4) and M13 (cluster 6) fingerprint profiles (Figure 2). The results obtained by MLST indicate that ST848 (n=5) isolates were isolated in the months of June (n=3) and November (n=2). It can be seen that ST848 isolates harbouring OXA-66 are clonal in nature as they clustered together in all of the three typing methods with 100% similarity pattern in case of DAF4 and M13 PCRs whereas 85% similarity in case of ERIC PCR. The timeframe indicates that this clone persisted over time from June as it was also observed in November. It can be said that ST862 isolates are different clones as one

strain has OXA-144 (AB12, June 2016) and the other has OXA-104 (Ab14, November 2016), further analyses using whole genome MLST can provide a better understanding on the evolution and stability of these strains. AB14 isolate was a ST862 strain whereas AB15 had ST1335 strain type. These two strains have differences in 3 housekeeping genes (Oxford MLST scheme) when compared to each other and M13 PCR rightly indicates that they are related but not identical to each other as opposed to DAF4 and ERIC PCR which cluster these two isolates at 100% similarity level. AB5 (ST218) and AB8 (ST195) had 1 allele difference at the MLST level and clustered well with DAF4 and ERIC PCR as opposed to M13 PCR. Similar clustering was also seen in the case of AB1 (ST1305) and AB3 (ST1306) but all 7 alleles were different and strains belonged to different clonal complexes. So different DNA fingerprinting techniques can often give different results which need to be supplemented with other genotypic and sequence based data for understanding the spread and persistence of clones in hospital settings.

252 **Discussion:**

253 Restriction and hybridisation based techniques like ribotyping and Pulsed-Field Gel
254 electrophoresis (PFGE) though have moderate – high relative discriminatory power, relative
255 reproducibility and repeatability; they are laborious and more time consuming not so cost
256 effective for routine surveillance in low income countries and requires special technical skills
257 such as DNA plug preparation and handling [15]. Multi-locus sequence typing (MLST) has a
258 high discriminatory power whereas Next generation sequencing (NGS) has a very high
259 discriminatory power but as these techniques involve multiple steps such as PCRs of
260 housekeeping gene and sequencing in case of MLST and library preps, genome assemblies
261 for NGS. MLST is a good epidemiological tool for global tracking of clones as opposed to
262 PFGE which is reliably detects hospital outbreaks and is useful in strain tracking related to
263 persistent hospital clones [31].

264 In a recent study, wgMLST (whole genome Multi Locus Sequence Typing) was regarded as
265 the best approach for real-time surveillance as it delivers optimal resolution and
266 epidemiological concordance while providing unambiguous nomenclature [36]. The Single
267 locus based typing (SBT) which involves amplifying and sequencing *bla*_{OXA-51-like} gene of *A.*
268 *baumannii* provides some advantage over MLST as it involves amplification and sequencing
269 of just one gene as opposed to seven and is said to distinguish all epidemic and sporadic
270 lineages of *A. baumannii* strains on par with MLST. Based on the findings of Pournaras *et al*
271 [33], SBT should correlate well with MLST but in our study it gave discrepant results where
272 *bla*_{OXA-66} (AB03), *bla*_{OXA-104} (AB14) and *bla*_{OXA-144} (AB12) clustered in a single clonal complex
273 862 (Table 1) and hence using SBT data alone can often lead to misinterpretation of the
274 results in order to distinguishing clones of *A. baumannii*. The clonal complex of strains in

275 comparison to the *bla*_{OXA-51-like} variants showed good correlation. Hence, it can be said that
276 SBT can give some information about the clone type and its association with Global Clones
277 1-8 but this has to be supplemented with other methods such as M13 PCR, PFGE, MLST or
278 whole genome MLST to reliably interpret the results.

279 Typing methods such as Rapid Amplified Polymorphic DNA (RAPD) typing (using M13, DAF4
280 primers) and repetitive extragenic palindrome (rep) PCR typing are simple and easy to
281 perform and can provide an effective alternative in understanding the epidemiology of
282 strains in resource limited countries as on most occasions they are reliable, less
283 complicated, rapid and cost effective [37]. Although it can have some disadvantages such as
284 low reproducibility between the labs but this can be standardised for usage within the same
285 lab [15]. With the three DNA fingerprinting methods such as M13, ERIC-2 and DAF4, we
286 observed that each technique produced a unique DNA fingerprint but M13 PCR was slightly
287 more reproducible with distinct bands than others and had a higher discriminatory power
288 than ERIC-2 and DAF-4 PCR techniques. Though the reproducibility of M13 technique may
289 be quiet low between laboratories due to several factors such as standardisation of
290 template concentration, use of PCR kits or enzymes used in reaction, thermocyclers and PCR
291 conditions [38], but within a single setting it can prove to effective in detecting persistent
292 clones that are may be difficult to detect in routine environmental surveillance where
293 environmental surveillance fails in detection of clones [34]. We also observed that M13 PCR
294 also had a higher discriminatory power than MLST and SBT (Figure 2), but small sample size
295 and using MLST as the gold standard was a limitation of our study. It has also been shown
296 previously that rep-PCR (ERIC) may prove to be less discriminatory between strains as a
297 result of repeated independent DNA preparations or DNA quality [39]. MLST data showed

that isolates belonging to Global clone 2 (GC2) (CC92 -Oxford scheme) found to occur in Asian countries and also in the Tamil Nadu region of South India was also the most prevalent clone in our study [40, 41]. On the contrary Global clone 1 (GC1) which corresponds to CC108 and found to be more prevalent in India was not observed in our study [40, 41].

The secondary finding of our study was detection of carbapenem resistance in *A. baumannii*. Previous studies on ESBLs have demonstrated, *bla*_{PER} was common and *bla*_{TEM} was rarely detected in *A. baumannii* [42, 43]. Similarly, in this study, *bla*_{PER-7} was detected in 10 isolates, whereas *bla*_{TEM-1} was found in only one isolate. It is indeed possible that the *bla*_{PER-7} gene could have been plasmid associated as observed by Opazo *et al* but this was not tested in the present study [43]. Pathogens that produce carbapenemase along with ESBL can be particularly challenging for clinicians to treat, since they are resistant to antibiotics of different classes [25]. In the present study, most of the isolates were positive for *ISAb_a1* and this element was also detected upstream of the *bla*_{OXA-51-like} and *bla*_{OXA-23-like} genes which suggests that carbapenem resistance may be due to the overexpression of *bla*_{OXA-51-like} and *bla*_{OXA-23-like} genes [30]. In one isolate (AB03, ST1306 isolated from blood and ETA of the same patient), the *bla*_{OXA-23-like} was bracketed by two copies of *ISAb_a1* forming a composite transposon (Tn2006) which has the potential to mobilise OXA-23 gene in different strains of *A. baumannii* [44]. Tn2006 occurring in different genotypic variants of *A. baumannii* is often associated with higher mortality [45] and this was true in the case of this patient who succumbed to his injuries during the course of this study.

The once predominant dogma that described antibiotic resistance and fitness as an inverse relationship may not hold true for Gram negative bacteria such as *Pseudomonas* where

increased virulence is associated with increased resistance to polymyxin B, gentamicin and ciprofloxacin as a result of swarming motility but this dogma holds true for bacteria such as *A. baumannii* [46]. The *cpa* gene known for degrading the coagulation factors like Factor V and fibrinogen, thus interfering with contact-activated clot formation in human plasma, a process which is required for effective innate immune defence during *A. baumannii* infection was observed in one isolate in our study [9]. By this disruption of the common pathway of coagulation the bacterial pathogens can promote bleeding and dissemination during infection in debilitated patients. Most of our isolates were positive for *pkf*, a serine protease responsible for degrading essential complement compounds such as C3 and complement regulators like properdin contributing to serum resistance and reduce biofilm formation, possibly by preventing steps involved in initial attachment. PKF plays an important role in *A. baumannii* serum resistance by the cleavage of complement components, leading to the dissociation of the cascade and subsequent survival of the bacterium [8]. *A. baumannii* shows pathology most often to patients who are severely immunocompromised, thus inactivation of the complement immune defence could allow an onset of fulminating infection. Proteases cleave proteins involved in cell-to-cell adhesion, which often results in dispersal of bacteria from a biofilm [47]. *A. baumannii* may be able to regulate its behaviour in a host by forming biofilm during optimal conditions or by producing PKF to inhibit biofilm formation when it would be advantageous for it to spread at different sites.

342 **Conclusions:**

343 We have compelling evidence that most of the *A. baumannii* infections are hospital acquired
344 especially in the ICU setting as evidenced by other studies. Due to the stringent sterilization
345 protocols most of the clones can be eradicated, but few persistent ones remain. Although
346 our sample size is quite low (n=17), and a single centre experience study from a single
347 department, it shows that M13 PCR is a cost effective technique which proves to be useful for
348 identification of clones and is slightly better than the DAF or ERIC PCR fingerprinting and
349 standardising this method can offer an alternative and a reliable way for strain tracking in a
350 routine lab environment which can help in prevention of further nosocomial outbreaks.
351 Fingerprinting analyses along with MLST or indeed whole genome MLST can further aid in
352 long term surveillance and in understanding bacterial strain evolution. Strict preventive
353 measures that reduce transmissibility of strains, along with routine surveillance practices,
354 can help decreasing patient morbidity and mortality and in improving antibiotic policies in
355 the hospital which in turn can slower the evolution of more resistant organisms.

356

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361 **Author Disclosure Statement**

362 No competing financial interests exist.

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Figure Legends

Figure 1: eBURST analysis for *Acinetobacter baumannii* obtained by comparing database STs against the studied STs (2017-08-16).

Figure 2: DNA finger printing pattern obtained - using a) DAF4, b) ERIC 2 and c) M13 primer; Lane 1-17 represents isolate AB01 to AB17 respectively.