An insight into the emergence of Acinetobacter baumannii as an oro-dental pathogen and its drug resistance gene profile — An in silico approach

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Abstract

Background: Acinetobacter baumannii, a potential nosocomial pathogen has stealthily gained entry into the oral cavity. Their association with other pathogens like Pseudomonas aeruginosa in chronic and aggressive periodontitis cases is well documented. The magnitude of problem caused by A. baumannii could be attributed to resistance genes acquired by the organism. Since the microbiome of oral cavity is heterogeneous and complex, the transfer of genes from multidrug resistant A. baumannii may be a serious threat in infection control and management. In view of this fact, the present study aims to categorize and characterize drug resistant genes present in each of the 19 genomes of Acinetobacter Sp. selected for the study.

Methods: About 19 genome sequences of Acinetobacter spp. with the predominance of different strains of A. baumannii was genotyped using in silico restriction digestion and pulse field gel electrophoresis (PFGE). Further, the
prevalence of common drug resistant genes in the genome of various *Acinetobacter* spp. was recorded using *in silico* PCR analysis.

**Results:** Based on the PFGE pattern, phylogenetic tree was constructed and the genomes were clustered into 6 genotypes. Genotype 4 (n = 8; 42.10%) and 5 (n = 6; 31.57%) were predominant, followed by genotypes 2 (n = 2; 10.52%), 1, 3 and 6 (n = 1; 5.26%). Three species were excluded from the list since they were negative for most of the drug resistant genes tested. Prevalence of drug resistant genes in each of the 16 genomes analysed found *oxa-51*, *ISAba 1* and *ADC 1* to be the major genes found in *A. baumannii*. *Acinetobacter* spp. belonging to genotypes 4 and 5 were found to harbour 6–10 and 2–8 potential drug resistant genes respectively.

**Conclusion:** The present study showed cluster of multi-drug resistant genes in genomes analysed, thus, warranting the need for antibiotic surveillance, alternate therapeutic measures and development of novel antimicrobials. An extensive study on the genes conferring drug resistance in this pathogen will open new avenues for battling the entry and spread of this pathogen in vulnerable patient groups.

Keywords: Microbiology, Genetics

### 1. Introduction

*A. baumannii*, a Gram-negative coco-bacilli has established itself as the most successful nosocomial pathogen within a short span of time with 2–10% of mortality rate recorded among patients with chronic urinary tract infections, bacteremia, pneumonia and critically ill patients in ICU [1]. The World Health Organization [WHO] have provided a red alert about the carbapenem resistant *A. baumannii* which secures its place under the “critical” category [2, 3]. An infamous fact about *A. baumannii* is its rapid evolution from multi-drug resistant [MDR] to extensively drug-resistant [XDR] form which has recently been escalated to pan-resistant [PDR] status [4]. Innate resistance together with the ability to accommodate extrinsic resistance factors has contributed to the resurgence of this pathogen with utmost potential [5].

The pathogen has been identified with much greater frequency in endodontic infections [6] along with other known dental pathogens. Prevalence of *A. baumannii* among patients with chronic or aggressive periodontitis is reported to be higher compared to the control group [7, 8]. The ability to form biofilm and resist desiccation is a key property of *A. baumannii* which makes it refractory to endodontic and periodontal treatments. The biofilms produced by the bacteria are also potential reservoirs of pathogens associated with pneumonia and chronic obstructive pulmonary disease [9, 10]. In a polymicrobial community, *A.*
*baumannii* protects carbapenems-susceptible bacteria, thus aggravating the disease process during treatment with carbapenems [11]. Although reports on *A. baumannii* as a dental pathogen is minimal, the propensity of the pathogen to evolve with a drug resistant armour underscores the need for more research on this pathogen and its role in oral infections. Awareness about the evolution of this pathogen and its spread in the community settings should be monitored to avoid sudden outbreak.

The present study on this nosocomial pathogen aims to comprehend the drug resistant genes [DR] in the genomes of *Acinetobacter* sp. using *in silico* tools. Although the strains used in the study are not representatives of *Acinetobacter* sp. from the oral cavity, they represent a small subset of the multi-drug resistant pathogen evolving in the community. Hence, the *in silico* analysis delimits to the information that is available in the database and provides an alert on the growing concerns about emerging pathogens in dentistry. An extensive epidemiological investigation including samples from dental specimens is required to prove the role of *A. baumannii* in the development of oral diseases.

2. Methods

2.1. Strains used in the study

Genomes of strains used in the present study as retrieved from NCBI [National Centre for Biotechnology Information] database are given in Table 1. A randomized subset comprising of nineteen isolates of genus *Acinetobacter* available in the database of *in silico* simulation tools for molecular biology experiments [http://insilico.ehu.es/PCR/] [12, 13] were used for drug resistant gene profiling.

2.2. PCR amplification

Primers for thirty seven commonly reported drug resistant genes were selected for the study and are summarized in Table 2. *In silico* characterization and amplification of resistant genes were performed using *in silico* simulation tools for molecular biology experiments [12, 13].

2.3. Pulse field gel electrophoresis [PFGE]

PFGE digestion of the selected genome was carried out using *Apa I* restriction enzyme, which recognizes the site 5’-GGGCC-3’ to produce cohesive end cleavage. The band pattern obtained after PFGE analysis was used to construct the phylogenetic tree [12, 13]. Lambda DNA ladder was used as a reference standard.
3. Results

3.1. Species confirmation

*Acinetobacter baumannii* was confirmed using 16S-23S ribosomal DNA intergenic spacer region which produced an amplicon size of 208bp upon *in silico* amplification using appropriate primers [Table 2]. Out of 19 genomes selected 16 were confirmed as *Acinetobacter baumannii*, one as *A. pitti* (G6) [NC_016603], one as *A. oleivorans* aka *Acinetobacter sp. DR1* (G1) [NC_014259] and one as uncharacterized *Acinetobacter sp. ADP1* (G2) [NC_005966] [Table 1].

3.2. Phylogenetic analysis

PFGE was used for the construction of the phylogenetic tree. The restriction enzyme *Apa I* was used to cleave genomic DNA sequence of the selected strains and the dendrogram was obtained from the band patterns. Six genotypes [G1- G6] were classified based on strain similarities and were clustered into each of the genotypes [Fig. 1]. Among the 6 genotypes [G], G4 was found to be the major genotype.

### Table 1. Genome sequences of *Acinetobacter* sp. used in the present study.

| S.NO | RefSeq     | Species of *Acinetobacter*                     | Genome size (Mb) | Genes |
|------|------------|------------------------------------------------|-------------------|-------|
| 1.   | NC_014259  | *Acinetobacter* sp. DR1                         | 4.12              | 3999  |
| 2.   | NC_005966  | *Acinetobacter* sp. ADP1                        | 3.59              | 3359  |
| 3.   | NC_010400  | *Acinetobacter baumannii SDF*                   | 3.42              | *     |
| 4.   | NC_017387  | *Acinetobacter baumannii TCDC-AB0715            | 4.13              | *     |
| 5.   | NC_017162  | *Acinetobacter baumannii 1656-2 chromosome      | 3.9               | 3922  |
| 6.   | NC_010611  | *Acinetobacter baumannii ACICU*                 | 3.9               | 3839  |
| 7.   | NC_017847  | *Acinetobacter baumannii MDR-TJ*                | 3.96              | 4071  |
| 8.   | NC_021726  | *Acinetobacter baumannii BJAB07104*             | 3.95              | 3910  |
| 9.   | NC_017171  | *Acinetobacter baumannii MDR-ZJ06*              | 3.99              | 3882  |
| 10.  | NC_021729  | *Acinetobacter baumannii BJAB0868*              | 3.90              | 3861  |
| 11.  | NC_018706  | *Acinetobacter baumannii TYTH-1*                | 3.95              | 3795  |
| 12.  | NC_021733  | *Acinetobacter baumannii BJAB0715*              | 4.00              | 3918  |
| 13.  | NC_009085  | *Acinetobacter baumannii ATCC 17978*            | 3.97              | *     |
| 14.  | NC_010410  | *Acinetobacter baumannii AYE*                   | 3.93              | 3900  |
| 15.  | NC_011595  | *Acinetobacter baumannii AB307-0294*            | 3.76              | 3544  |
| 16.  | NC_011586  | *Acinetobacter baumannii AB0057*                | 4.05              | 3971  |
| 17.  | NC_023028  | *Acinetobacter baumannii ZW85-1*                | 3.76              | 3712  |
| 18.  | NC_020547  | *Acinetobacter baumannii D1279779*              | 3.70              | 3564  |
| 19.  | NC_016603  | *Acinetobacter pitti PHEA-2*                    | 3.86              | 3674  |

* Data not available.
### Table 2. Primers used in the present study.

| Target          | Primer sequence (5’-3’)                                      | Amplicon size (bp) | Reference                |
|-----------------|-------------------------------------------------------------|--------------------|--------------------------|
| **16S-23S rRNA ITS region** | F: CATTATCACGGTAATTTAGTG  
R: AGAGGCACGTGCACTTAAAG | 208                | Chen et al., 2014 [38]  |
| **blaOxa-23 like** | F: GATCGGATTGGAGAACCAGA  
R: ATTTCTGACCGCATCTACAC | 501                | Woodford et al., 2006 [39]  |
| **blaOxa-24 like** | F: GGTGATTGCCCCCTTAAA  
R: AGTGGACGAAAAGGGGATT | 246                | Woodford et al., 2006 [39]  |
| **blaOxa-51 like** | F: TAAATGTTGTGATCGCCTTG  
R: TGGAATGGCCTACTCATCTTGG | 353                | Woodford et al., 2006 [39]  |
| **blaOxa-58 like** | F: AAGTATTGGGCGCTTGGGCTG  
R: CCCCTCGCGCTACGTACAC | 599                | Woodford et al., 2006 [39]  |
| **blaOxa-143 like** | F: TGCGACTTTTCGACGTCTTCT  
R: TAACTCTGAGGGGCAACC | 149                | Higgins et al., 2010 [40]  |
| **blaVIM** | F: GTTGTTGCGATATCGCAAC  
R: AATGCACAGCACCAGGATAG | 382                | Mendes et al., 2007 [41]  |
| **blaIMP** | F: GAATAGAATGGTTAACTCTC  
R: CCAAACCACTACGTATCTC | 188                | Mendes et al., 2007 [41]  |
| **blaGIM** | F: TCAATTAGCTCTTGCGCTGAC  
R: CGGAACAGCACCAGGATAG | 72                 | Mendes et al., 2007 [41]  |
| **blaNDM-1** | F: GGTGGGCGATCTGTCACTTGC  
R: CGGAATGGCCTACTCATCTTGG | 621                | Nordmann et al., 2011 [42]  |
| **ISAba-1** | F: ATGCACGCGCTTCTTGCGAC  
R: AATGATTGGGACATCGAAG | 393                | H’eritier et al., 2006 [43]  |
| **ISAba-4** | F: ATGTAAGACCTACTGATGGGC  
R: ACTCTCATATTATTTTCTGG | 611                | Corvec et al., 2007 [44]  |
| **ISAba-125** | F: GGGTAATGCTGTATGCTG  
R: TAGACTAGCAGCGCATGCA | 148                | Lopes and Amyes, 2012 [19]  |
| **blaTEM** | ATGATGATCAGGAAGGTCACCCAGC  
CCAATGTTAATCGATGAG | 858                | Verdet et al., 2006 [45]  |
| **blaSHV** | TATCTCTCTTGTAGCCACC  
GATTGCGATTTGCGCTCTCG | 795                | Verdet et al., 2006 [45]  |
| **blaCTX-M** | CGCTTTGCGATGTGCAG  
ACCGCGATATCGTTGGT | 550                | Messai et al., 2008 [46]  |
| **blaPER** | ATGAATGTCATTATAAAAGC  
AAATTGGCGTACAGGCAAG | 925                | Kim et al., 2004 [47]  |
| **blaVEB** | CGACTTACTTTCGATGGCAGC  
GGACTCTGCAACAAATACGC | 643                | Kim et al., 2004 [47]  |
| **blaGES** | ATGCGCTTCATCATCACGCAC  
CTATTTGCTGGTGGTT | 860                | Kim et al., 2004 [47]  |
| **blaADC** | CCGCGACACAGGATGGTTGAG  
TGCGCGATATTTTCTGGT | 420                | Ruiz et al., 2007 [48]  |
| **qnr A** | AGAGGTATTTCTCACGCGAGG  
TGCCAGGCGACGATCTTGAC | 580                | Figueira et al., 2011 [49]  |
| **qnr B** | GGMATHGAAATTGCCTACGT  
TTTGGYCCAGCGATGGGA | 246                | Figueira et al., 2011 [49]  |
| **qnr S** | GCAAGTTTCAAGACAGGGTG  
TCTAAACCAGGTCAGTGCTGG | 428                | Figueira et al., 2011 [49]  |

(continued on next page)
### 3.3. PCR amplification of drug resistant genes

*In silico* amplification of common drug resistant genes [DR] revealed that out of 37 DR gene analysed, 15 genes showed the presence of amplicons distributed among 4 genotypes [2, 3, 4, 5]. *blaOxa-51* was found to be the most prevalent gene in G4 [50%], G5 [37.5%] and G3 [6.25%] among all the 16 genomes analysed. Interestingly, *Acinetobacter* sp. belonging to G3, G4 and G5 genotypes were positive for *blaOxa-51* [100%]. *ISAba 1* and *ADC 1* were also found to be present in all the strains.

| Target |
| :----- |
| Primer sequence (5'-3') |
| Amplicon size (bp) |
| Reference |
| **aac6(Ib)** |
| TTGGCAGTCTCTATGAGTGGCTA | 482 |
| CTGCAATTGCGCTGGCGCTTT |
| Figueira et al., 2011 [49] |
| **tet A** |
| GTAATTCTGAGCAGTCTGCG | 954 |
| CTGCTGAGCAACATTGCTT |
| Guardabassi et al., 2000 [50] |
| **tet B** |
| CTAGGATATCGTGTTTGTG | 414 |
| ACTCCCCGAGCCTGGG |
| Guardabassi et al., 2000 [50] |
| **intI 1** |
| CCTCCGAATGTGTAACCGC | 248 |
| AGCCCTGAGCAGGAAATAC |
| Murinda et al., 2005 [51] |
| **qacEΔ1** |
| GAGGGCTTATGACCGTGC | 200 |
| ATACCTACAAAGGCCCAACGC |
| Murinda et al., 2005 [51] |
| **sul 1** |
| TCAGAGCTGTTGATCGCG | 346 |
| GAAAGAACCGCACAAATCTC |
| Murinda et al., 2005 [51] |
| **sul 2** |
| GCCGTCAGGAGATGGGATT | 293 |
| GCAGTTGATACGGCAGGC |
| Frank et al., 2007 [52] |
| **sul 3** |
| GGAAGAAATCCAAAAGACTCAAC | 363 |
| CCTAAAAAGAAGCCCATACC |
| Frank et al., 2007 [52] |
| **intI 2** |
| GTGCAAACGAGTGGACGAAATG | 789 |
| CAGGATATGGCAGAAAAAGGT |
| Valenzuela et al., 2007 [53] |
| **dfr A1** |
| GTGAAACTATCCTGATAGG | 471 |
| ACCCTTGGCCAGATTTT |
| Seputiene et al., 2010 [54] |
| **dfr A5** |
| GCBAAGGDDGARCACT | 394 |
| TTTMCCAYATTTGATAGC |
| Seputiene et al., 2010 [54] |
| **dfr A7** |
| AAATTTCCATTATTCTGCA | 471 |
| TTAGCCTTTTTCCAAATTC |
| Seputiene et al., 2010 [54] |
| **dfr A8** |
| TTGGGAAGGACGCACCTT | 382 |
| ACCATTCCGACGATCAA |
| Seputiene et al., 2010 [54] |
| **dfr A12** |
| GGTGAGGARAAATTTCTGAC | 309 |
| TGGGAAGGCGTCCACCCCTC |
| Seputiene et al., 2010 [54] |
| **carO** |
| AAAGTATTACGGTGGTTGATG | 750 |
| TTACCAGTAGAGTTTACC |
| Mussi et al., 2005 [55] |

= 8; 42.1%] followed by G5 [n = 6; 31.57%], G2 [n = 2; 10.52%], G1, G3, G6 [n = 1; 5.26% each] [Fig. 1].
of *A. baumannii* belonging to G4. *qacEΔ1*, *sul 1* and *sul 2* were predominant in G4 with a prevalence of 43.75%, 43.75%, and 37.5% respectively [Table 3]. About 12 different genes coding for drug resistance have been found in G4 and 5. *A. baumannii* [NC_021726] harbours the highest number of resistant genes when compared to the other isolates [Table 4]. *In silico* amplification of DR genes revealed that *A. pitti* [NC_016603], *A. oleivorans* aka *Acinetobacter* sp. DR1 [NC_014259] and uncharacterized *Acinetobacter* sp. ADP1 [NC_005966] did not produce any amplicon for the panel of genes selected for the study [Fig. 2].

### 4. Discussion

When reports on methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis* and multi-drug resistant *Pseudomonas aeruginosa* occupied a greater sector of the hospital acquired and community-based infections, a more potent pathogen emerged with mammoth abilities to combat almost all antibiotics of different generations. *Acinetobacter baumannii*, the critical pathogen, which was once considered to be opportunistic, has stealthily entered the oral cavity. Ignoring this pathogen and its drug-resistant gene repository would pose a serious threat while treating patients with soft tissue infections of the oral cavity. In line with the above facts, the present study was designed to characterize *Acinetobacter* sp. based on their PFGE pattern into genotypes and attribute frequencies of DR genes in each of the classified genotypes using computational tools. The results of 16S-23S ribosomal DNA intergenic spacer region [ITS] amplification was used to differentiate *A. baumannii* from other species such as *A. pitti*, *A. oleivorans* etc., which was coherent with the genotypic identification of *Acinetobacter* sp. from clinical samples [14]. The present in silico study showed 100% accuracy in detecting amplicons of ITS region specific for *A. baumannii*. PFGE was carried out to classify genotypes, which returned 2 major [G4 and G5] and 4 minor genotypes [G1, 2, 3, 6].
Development of resistance in pathogens to antimicrobials is threatening mankind. Intrinsic and extrinsic mechanisms act together to make the pathogen more and more potent against most of the available therapeutic drugs. Common drug resistant mechanisms identified in A. baumannii is associated with carbapenemase production. Beta-lactamases [Ambler class A] and oxacillinases [class D] are two major

Table 3. Frequency and distribution of drug resistant genes in the genotypes classified.

| Genes   | Genotype | Specific genotype content | Frequency of DR gene | Genotype based Percentage (%) | Overall Percentage (%) |
|---------|----------|---------------------------|----------------------|-------------------------------|------------------------|
| blaoxa-23 | 4        | 8                         | 5                    | 62.5                          | 31.25                  |
|         | 5        | 6                         | 1                    | 16.7                          | 6.25                   |
| blaoxa-51 | 3        | 1                         | 1                    | 100                           | 6.25                   |
|         | 4        | 8                         | 8                    | 100                           | 50                     |
|         | 5        | 6                         | 6                    | 100                           | 37.5                   |
| blaoxa-58 | 4        | 8                         | 2                    | 25                            | 12.5                   |
| ISAba1   | 4        | 8                         | 8                    | 100                           | 50                     |
|         | 5        | 6                         | 3                    | 50                            | 18.75                  |
| ISAba125 | 3        | 1                         | 1                    | 100                           | 6.25                   |
|         | 4        | 8                         | 2                    | 25                            | 12.5                   |
| blaveB   | 5        | 6                         | 1                    | 16.7                          | 6.25                   |
| blaadC 1 | 4        | 8                         | 8                    | 100                           | 50                     |
|         | 5        | 6                         | 5                    | 83.3                          | 31.25                  |
| aac6(Ib) | 3        | 1                         | 1                    | 100                           | 6.25                   |
|         | 4        | 8                         | 6                    | 75                            | 37.5                   |
| tet A    | 5        | 6                         | 2                    | 33.3                          | 12.5                   |
| tet B    | 3        | 1                         | 1                    | 100                           | 6.25                   |
|         | 4        | 8                         | 5                    | 62.5                          | 31.25                  |
|         | 5        | 6                         | 1                    | 16.7                          | 6.25                   |
| qaceA1   | 3        | 1                         | 1                    | 100                           | 6.25                   |
|         | 4        | 8                         | 7                    | 87.5                          | 43.75                  |
|         | 5        | 6                         | 2                    | 33.3                          | 12.5                   |
| sul 1    | 3        | 1                         | 1                    | 100                           | 6.25                   |
|         | 4        | 8                         | 7                    | 87.5                          | 43.75                  |
|         | 5        | 6                         | 2                    | 33.3                          | 12.5                   |
| sul 2    | 4        | 8                         | 6                    | 75                            | 37.5                   |
|         | 5        | 6                         | 2                    | 33.3                          | 12.5                   |
| dfra1    | 5        | 6                         | 1                    | 16.7                          | 6.25                   |
| carO     | 2        | 1                         | 1                    | 100                           | 6.25                   |
|         | 4        | 8                         | 1                    | 12.5                          | 6.25                   |
|         | 5        | 6                         | 2                    | 33.3                          | 12.5                   |
groups of enzymes responsible for resistance to carbapenems which is most commonly and abundantly used drug in healthcare settings. \textit{bla}\textsubscript{Oxa-51-like} and \textit{bla}\textsubscript{Oxa23-like} are reported in earlier studies as dominant genes prevalent in \textit{A. baumannii} \cite{15, 16, 17, 18}. The present study also provides similar findings wherein 50\% \([n = 8]\), 37.5\% \([n = 6]\) and 6.25\% \([n = 1]\) of \textit{A. baumannii} isolates belonging to G4, G5 and G3 exhibited the presence of \textit{bla}\textsubscript{Oxa-51-like} genes respectively. An interesting

| S.NO | RefSeq     | Species of Acinetobacter                  | Number of PCR positive DR genes | Genotype 2 | Genotype 3 | Genotype 4 | Genotype 5 |
|------|------------|------------------------------------------|---------------------------------|------------|------------|------------|------------|
| 1    | NC_010400  | Acinetobacter baumannii SDF              | 1                               |            |            |            |            |
| 2    | NC_017387  | Acinetobacter baumannii TCDC-AB0715      | 6                               |            |            |            |            |
| 3    | NC_017162  | Acinetobacter baumannii 1656-2 chromosome| 6                               |            |            |            |            |
| 4    | NC_010611  | Acinetobacter baumannii ACICU            | 8                               |            |            |            |            |
| 5    | NC_017847  | Acinetobacter baumannii MDR-TJ           | 9                               |            |            |            |            |
| 6    | NC_021726  | Acinetobacter baumannii BJAB07104        | 10                              |            |            |            |            |
| 7    | NC_017171  | Acinetobacter baumannii MDR-ZJ06         | 8                               |            |            |            |            |
| 8    | NC_021729  | Acinetobacter baumannii BJAB0868         | 9                               |            |            |            |            |
| 9    | NC_018706  | Acinetobacter baumannii TYTH-1           | 8                               |            |            |            |            |
| 10   | NC_021733  | Acinetobacter baumannii BJAB0715         | 8                               |            |            |            |            |
| 11   | NC_009085  | Acinetobacter baumannii ATCC 17978       | 4                               |            |            |            |            |
| 12   | NC_010410  | Acinetobacter baumannii AYE              | 8                               |            |            |            |            |
| 13   | NC_011595  | Acinetobacter baumannii AB307-0294       | 2                               |            |            |            |            |
| 14   | NC_011586  | Acinetobacter baumannii AB0057           | 7                               |            |            |            |            |
| 15   | NC_023028  | Acinetobacter baumannii ZW85-1           | 5                               |            |            |            |            |
| 16   | NC_020547  | Acinetobacter baumannii D1279779         | 2                               |            |            |            |            |
finding in this study is that A. baumannii strain SDF was found to be PCR negative for an intrinsic \( bla_{Oxa-51-like} \) gene. The probable reason might be the fact that this strain was isolated from the human body lice and is susceptible to almost all antibiotics [19]. \( ISAba1 \) belongs to class 1 integrons which is capable of transferring gene cassettes from one organism to the other. Interestingly, the presence of \( ISAba1 \) upstream of \( bla_{Oxa-51-like} \) is known to increase the expression of the encoded enzyme by acting as a strong promoter. In the present study, 100% of strains belonging to G4 and 50% of G5 were found to possess \( ISAba1 \). \( bla_{Oxa-23-like} \) was found in majority G4, G3 and one strain of G5, whereas \( bla_{Oxa58-like} \) was found in a few strains of G4.

\( ampC \) type of beta-lactamase is naturally produced by Gram-negative bacteria. The enzyme is non-inducible and produced only at very low levels. In the presence of insertion elements, expression of \( ampC \) increases with the dissemination of genes thereby elevating the organism to a resistant state. The \( bla_{ADC} \) [\( Acinetobacter \) derived cephalosporinases] gene encodes \( ampC \) \( \beta \)-lactamase, which in the presence of insertion elements \( ISAba1 \) or \( ISAba125 \), overproduces the enzyme. A comparative study on the placement of \( ISAba1 \) or \( ISAba125 \) on the upstream of \( bla_{ADC} \) was carried out. It was found that expression of \( bla_{ADC} \) was 6-times more when compared to constitutively expressed \( bla_{ADC} \) with no upstream insertion element. The study also suggested the formation of hybrid gene which combines -10 promoter sequence of \( ISAba125 \) and -35 region of \( bla_{ADC} \) gene which conferred resistance to cephalosporins [20].

Numerous novel types of extended spectrum beta—lactamase have emerged globally from which VEB [for Vietnamese extended-spectrum beta—lactamase] family is one. The presence of \( bla_{VEB} \) has been inconsistent with high prevalence [21] recorded in certain geographical locations and completely absent in a few others [22]. The present study records only one strain [A. baumannii AYE] with \( bla_{VEB} \) \([n = 1, 16.7\%]\) in G5. Aminoglycoside-modifying enzyme [AME] encoded by \( aac6 \ [Ib] \) renders A. baumannii strains resistant to aminoglycosides including amikacin [23]. This gene is not only found in the chromosome but also found in plasmids, integrons, transposons, genomic islands etc., \( aac6 \ [Ib] \) was found in a few isolates in genotypes 3 \([n = 1, 100\%]\) and 4 \([n = 6; 75\%]\).

The DR genes \( tetA \) and \( tetB \) encodes factors specific to efflux pump proteins that scavenge tetracycline from the cells [24]. Tet genes are mostly plasmid encoded and can easily be transferred to other closely related species [25]. While \( tetA \) confers resistance towards tetra and doxycycline, \( tetB \) includes resistance to minocycline also [26]. Although \( tetA \) was found only in G5 \([n = 2; 33.3\%]\), \( tetB \) was more graciously distributed among G3 \([n = 1; 100\%]\), 4 \([n = 4; 50\%]\) and 5 \([n = 16.7\%]\) in the present study which were concordant with earlier report of Marti et al. 2006 [27], which recorded a prevalence of 13.6% of \( tetA \) and 66% of \( tetB \). Another group of efflux system which enables A. baumannii to survive in the
environment is the biocide efflux family, encoded by qac genes. qacEΔ1 genes are widely propagated and disseminated through plasmid-mediated class I integrons [28]. High prevalence of qacEΔ1 has been reported in clinical isolates of Acinetobacter making the organism resistant to both antibiotics as well as antiseptics [29]. The current study also records a high frequency of qacEΔ1 gene in the isolates analyzed, with 100%, 87.5% and 33.3% in genotypes 3, 4, and 5 respectively.

Sulfonamides, dihydrofolic acid inhibitor, are commonly used in veterinary and clinical settings to treat bacterial and protozoal infections. Three vital genes coding for sulphonamide resistance namely sul1 [30] sul2 [31] and sul3 [32] are located in the 3′-conserved region of class 1 integron and non-conjugative plasmids. All these genes were initially identified in pathogens dwelling in the soil environment. A recent study by Khorsi et al. 2015 [33] reported a high prevalence of sul1 [36.17%] and sul2 [77.65%] in clinical specimens which are in contrast to the findings of the present study with an overall frequency of 62.5% for sul1 and 50% for sul2. A similar gene involved in the folate synthesis dfrA, dihydrofolate reductase mediates drug resistance to trimethoprim. It is one of the few DR genes associated with class II integrons [34]. Akrami et al. [35] and Nourbakhsh et al., 2017 [36], reported the frequency of dfrA1 to be around 77.1% and 63.7% which is very high compared to scores obtained from the present study which is 6.25%. This inconsistency can be attributed to the type, geographical location, association with disease, etc., of the isolates used in the study.

carO is a carbapenem associated outer membrane protein [carO] which is a protein that selectively allows the uptake of amino acids and imipenem. Loss of membrane permeability [loss of carO] due to genetic alterations confers resistance towards the specific antibiotic imipenem [37]. Several studies have already established the modification of porins leading to antibiotic resistance. Zhao et al. 2015 [38] reported the prevalence of carO gene in clinical isolates of A. baumannii to be 64.2%. We report a much lesser frequency of carO, which was in the range of 6.25–12.5% distributed among various genotypes. Additionally, we found that three out of four strains with intact carO gene as detected by in silico PCR exhibited susceptibility (A. baumannii SDF) or resistance (A. baumannii ATCC 17978, D1279779) to only a few antibiotics (n = 2–4). In contrast, one strain, A. baumannii BJAB07104, which demonstrated PCR positivity for carO gene was found to harbour about ten genes encoding drug resistance. The paradox identified needs further investigation to justify the relationship between the integrity of membrane proteins and the mechanism of drug resistance.

The alarming increase in the number of drug-resistant genes acquired by A. baumannii has made a significant impact in the community settings. On the other hand, the transfer of genes from the pathogen to other commensals intensifies the problem in treating diseases. Several characteristics of A. baumannii such as biofilm formation,
ability to survive stressors, desiccation, makes it more suitable to gain entry into the oral cavity. Prosthetic devices, implants contaminated with the pathogen, may delay or even impede the treatment process. Since the oral cavity is loaded with a polymicrobial community, the ease of transfer of drug-resistant genes from one organism to other is remarkably high.

5. Conclusion

Intrinsic and extrinsic drug resistant mechanisms work parallely to produce a more stable and persistent strain of this pathogen. The remarkable drug-resistant profile of *A. baumannii* should not be overlooked as it may turn in to a greatest microbial menace in dentistry. Although the study addresses the major factors intended to be discussed, some of the limitations are [a] the phenotype and genotype correlation could not be performed due to lack of antimicrobial sensitivity data, [b] the sequences analysed by in silico method may not reflect the actual frequencies of DR genes in the clinical isolates of oral cavity, as the exposure and selective pressure influences the extrinsic drug-resistant phenotypes, [c] the frequencies of DR gene gives a crude estimate and does not represent all the emerging strains of *A. baumannii* and most importantly [d] these strains are not derived from oral infections. Nevertheless, this study with all its pros and cons focused has emphasized the fact that resurgence of antibiotic-resistant *A. baumannii* in community and emergence in dental settings could be a serious threat when ignored. Precautions should be taken to avoid the spread of this pathogen from the healthcare settings into the community or the environment.

Declarations

Author contribution statement

Vijayashree Priyadharsini Jayaseelan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Smiline Girija AS, Paramasivam Arumugam: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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