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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

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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 cocco-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.*

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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′-GGGCCC-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.

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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.

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 [n

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Table 2. Primers used in the present study.

Target	Primer sequence (5′-3′)	Amplicon size (bp)	Reference
16S-23S rRNA ITS region	F: CATTATCACGGTAATTAGTG R: AGAGCACTGTGCACTTAAG	208	Chen *et al.*, 2014 [38]
$bla_{Oxa\text{-}23\ like}$	F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCCAT	501	Woodford *et al.*, 2006 [39]
$bla_{Oxa\text{-}24\ like}$	F: GGTTAGTTGGCCCCCTTAAA R: AGTTGAGCGAAAAGGGGATT	246	Woodford *et al.*, 2006 [39]
$bla_{Oxa\text{-}51\ like}$	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACTTCATCTTGG	353	Woodford *et al.*, 2006 [39]
$bla_{Oxa\text{-}58\ like}$	F: AAGTATTGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	599	Woodford *et al.*, 2006 [39]
$bla_{Oxa\text{-}143\ like}$	F: TGGCACTTTCAGCAGTTCCT R: TAATCTTGAGGGGGCCAACC	149	Higgins *et al.*, 2010 [40]
bla_{VIM}	F: GTTTGGTCGCATATCGCAAC R: AATGCGCAGCACCAGGATAG	382	Mendes *et al.*, 2007 [41]
bla_{IMP}	F: GAATAGAATGGTTAACTCTC R: CCAAACCACTAGGTTATC	188	Mendes *et al.*, 2007 [41]
bla_{GIM}	F: TCAATTAGCTCTTGGGCTGAC R: CGGAACGACCATTTGAATGG	72	Mendes *et al.*, 2007 [41]
$bla_{NDM\text{-}1}$	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621	Nordmann *et al.*, 2011 [42]
ISAba-1	F: ATGCAGCGCTTCTTTGCAGG R: AATGATTGGTGACAATGAAG	393	H'eritier *et al.*, 2006 [43]
ISAba-4	F: ATTTGAACCCATCTATTGGC R: ACTCTCATATTTTTTCTTGG	611	Corvec *et al.*, 2007 [44]
ISAba-125	F: GGGTAATGCTCGTATCGT R: TAGACGTAGACGTGGTCA	148	Lopes and Amyes, 2012 [19]
bla_{TEM}	ATGATGATTCAACATTTCCG CCAATGCTTAATCAGTGAGG	858	Verdet *et al.*, 2006 [45]
bla_{SHV}	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTCGCTCGG	795	Verdet *et al.*, 2006 [45]
$bla_{CTX\text{-}M}$	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	550	Messai *et al.*, 2008 [46]
bla_{PER}	ATGAATGTCATTATAAAAGC AATTTGGGCTTAGGGCAGAA	925	Kim *et al.*, 2004 [47]
bla_{VEB}	CGACTTCCATTTCCCGATGC GGACTCTGCAACAAATACGC	643	Kim *et al.*, 2004 [47]
bla_{GES}	ATGCGCTTCATTCACGCAC CTATTTGTCCGTGCTCAGG	860	Kim *et al.*, 2004 [47]
bla_{ADC}	CCGCGACAGCAGGTGGATA TCGGCTGATTTTCTTGGTT	420	Ruiz *et al.*, 2007 [48]
qnr A	AGAGGATTTCTCACGCCAGG TGCCAGGCACAGATCTTGAC	580	Figueira *et al.*, 2011 [49]
qnr B	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	246	Figueira *et al.*, 2011 [49]
qnr S	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	Figueira *et al.*, 2011 [49]

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Table 2. (*Continued*)

Target	Primer sequence (5′-3′)	Amplicon size (bp)	Reference
aac6(Ib)	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	482	Figueira *et al.*, 2011 [49]
tet A	GTAATTCTGAGCACTGTCGC CTGCCTGGACAACATTGCTT	954	Guardabassi *et al.*, 2000 [50]
tet B	CTCAGTATTCCAAGCCTTTG ACTCCCCTGAGCTTGAGGGG	414	Guardabassi *et al.*, 2000 [50]
intI 1	CCTTCGAATGCTGTAACCGC ACGCCCTTGAGCGGAAGTATC	248	Murinda *et al.*, 2005 [51]
qacEΔ1	GAGGGCTTTACTAAGCTTGC ATACCTACAAAGCCCCACGC	200	Murinda *et al.*, 2005 [51]
sul 1	TCAGACGTCGTGGATGTCG CGAAGAACCGCACAATCTCG	346	Murinda *et al.*, 2005 [51]
sul 2	GCGCTCAAGGCAGATGGCATT GCGTTTGATACCGGCACCCGT	293	Frank *et al.*, 2007 [52]
sul 3	GGAAGAAATCAAAAGACTCAA CCTAAAAAGAAGCCCATACC	363	Frank *et al.*, 2007 [52]
intI 2	GTAGCAAACGAGTGACGAAATG CACGGATATGCGACAAAAAGGT	789	Valenzuela *et al.*, 2007 [53]
dfr A1	GTGAAACTATCACTAATGG ACCCTTTTGCCAGATTTG	471	Seputiene *et al.*, 2010 [54]
dfr A5	GCBAAAGGDGARCAGCT TTTMCCAYATTTGATAGC	394	Seputiene *et al.*, 2010 [54]
dfr A7	AAAATTTCATTGATTTCTGCA TTAGCCTTTTTTCCAAATCT	471	Seputiene *et al.*, 2010 [54]
dfr A8	TTGGGAAGGACAACGCACTT ACCATTTCGGCCAGATCAAC	382	Seputiene *et al.*, 2010 [54]
dfr A12	GGTGAGCARAAGATYTTTCGC TGGGAAGAAGGCGTCACCCTC	309	Seputiene *et al.*, 2010 [54]
carO	AAAGTATTACGTGTTTTAGTG TTACCAGTAGAAGTTTACACC	750	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].

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]. $bla_{Oxa\text{-}51}$ 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 $bla_{oxa\text{-}51}$ [100%]. *ISAba 1* and *ADC 1* were also found to be present in all the strains

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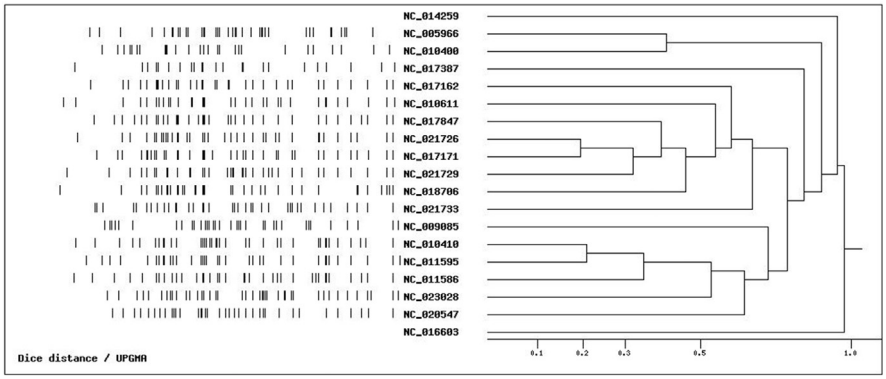

Fig. 1. Phylogenetic diversity of *Acinetobacter* sp. identified by *in silico* pulse field gel electrophoresis.

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].

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

Genes	Genotype	Specific genotype N	Frequency of DR gene	Genotype based Percentage (%)	Overall Percentage (%)
bla$_{oxa-23}$	4	8	5	62.5	31.25
	5	6	1	16.7	6.25
bla$_{oxa-51}$	3	1	1	100	6.25
	4	8	8	100	50
	5	6	6	100	37.5
bla$_{oxa-58}$	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
bla$_{VEB}$	5	6	1	16.7	6.25
bla$_{ADC\ 1}$	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
qacEΔ1	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

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

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Table 4. Number of drug resistant genes in each of the genotypes.

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				✓

groups of enzymes responsible for resistance to carbapenems which is most commonly and abundantly used drug in healthcare settings. $bla_{Oxa\text{-}51\text{-}like}$ and $bla_{Oxa23\text{-}like}$ are reported in earlier studies as dominant genes prevalent in *A. baumannii* [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 *A. baumannii* isolates belonging to G4, G5 and G3 exhibited the presence of $bla_{Oxa\text{-}51\text{-}like}$ genes respectively. An interesting

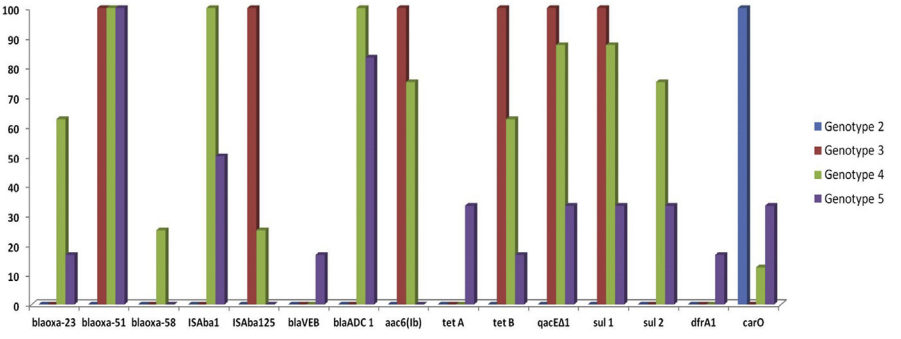

Fig. 2. Distribution of drug resistant genes in the strains belonging to different genotypes.

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finding in this study is that *A. baumannii* strain SDF was found to be PCR negative for an intrinsic $bla_{Oxa\text{-}51\text{-}like}$ 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 $bla_{Oxa\text{-}51\text{-}like}$ 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*. $bla_{Oxa\text{-}23\text{-}like}$ was found in majority G4, G3 and one strain of G5, whereas $bla_{Oxa58\text{-}like}$ 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* β-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

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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 *qacEΔ*1 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,

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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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Additional information

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