

BEYOND THE HOSPITAL: DETECTION AND CHARACTERIZATION OF VIRULENT *ACINETOBACTER BAUMANNII* IN RETAIL RAW MEAT

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ABSTRACT:

The present study was undertaken to determine the occurrence, antimicrobial resistance pattern, and virulence potential of *Acinetobacter baumannii* isolated from 195 meat samples (chicken, mutton, and beef) marketed in retail outlets in Chennai, India. From the 195 meat samples obtained for the study from retail outlets, 3.07% (6/195) were found to be positive for the presence of *A. baumannii* established by biochemical characterization and subsequently, PCR targeting species-specific *A.b_hyp* gene. Antimicrobial resistance profiling done by disc diffusion method revealed the highest prevalence of resistance against chloramphenicol and cefuroxime (100%) followed by ampicillin (50%) and ceftriaxone (33%) while 100% of the isolates were sensitive to cotrimoxazole, gentamicin, and tetracycline followed by 83.3% isolates showing sensitivity to ciprofloxacin. On genotypic characterization of antimicrobial resistance genes, it was found that all the isolates represented *bla_{OXA-51-like}* gene and *Int-1* gene while the *bla_{TEM}* gene was present in 50% (3/6) isolates. Out of the 6 isolates which were assessed, 83.3% of isolates showed the presence of motility, 66.67% showed hemolytic ability and 100% showed biofilm formation. All isolates showed the presence of genes encoding for virulence factors namely *bap*, *pgaB*, *csuE*, and *OmpA* genes, while *bla_{PER-1}* was exhibited by 4 (66.67%) of the *A.baumannii* isolates.

KEYWORDS: *Acinetobacter Baumannii*, Antimicrobial Resistance, Virulence, Meat, Food Safety, India

INTRODUCTION

Meat is considered a rich source of protein and nutrients and is consumed by many people throughout the world. The presence of antimicrobial-resistant ESKAPE pathogens in food products is a matter of global concern. Most of the foodborne diseases caused by ESKAPE pathogens are mainly linked to animal or plant-origin foods such as milk, meat, poultry, and vegetables or instigated from the food-related environment such as agricultural land, food industry, hospitals, kitchen, etc^[1]. Among ESKAPE pathogens over the past ten years, *Acinetobacter spp.* has universally emerged as a nosocomial pathogen with foodborne implications. Genus *Acinetobacter* belongs to the Moraxellaceae family, which comprises more than 59 classified species including *Acinetobacter baumannii*. The bacteria are short, pleomorphic, coccobacillary with Gram-negative orientation, strictly aerobic, catalase-positive, oxidase-negative, nonfermenting, and nonmotile organisms.

Acinetobacter baumannii, the most virulent *Acinetobacter* species, commonly causes hospital-acquired infections with high morbidity and mortality. But in recent times, *Acinetobacter baumannii* is been traced from a range of food items like meat, fruits, milk, milk products, and raw vegetables^[2]. This hints at a possibility of zoonotic transmission of this organism to humans through the food chain; which makes it essential for studies on this organism to be conducted from a food safety perspective.

Antimicrobial resistance is a serious threat today. *A. baumannii* readily develops resistance to many drugs through various mechanisms. Carbapenemase-producing *A. baumannii* is classified by the WHO as a Priority 1 - Critical Pathogen, highlighting the urgent need for new antibiotics. Organisms isolated from meat samples have

previously shown records of antibiotic resistance^[2]. *Acinetobacter* spp. is not only found to be resistant to major antibiotics like tetracycline, sulphonamide, erythromycin, trimethoprim, ciprofloxacin, etc but it is also involved in the spread of genes responsible for the resistance^[3]. Eating contaminated, especially raw or undercooked meat, promotes antimicrobial resistance in consumers. Infections with resistant bacteria cause longer recovery, ineffective antibiotics, treatment failure, and higher mortality.

In the past decade, many studies have explored the unique mechanisms behind *A. baumannii*'s success as a pathogen. It possesses several virulence factors, including hemolytic factors, efflux pumps, lipopolysaccharides, iron acquisition systems, and *OmpA*, which aids bacterial adherence to epithelial cells^[3]. Some virulence factors inhibit the host's immune response by promoting biofilm formation, colonising the epithelium, and obtaining nutrients from the host. Although traditionally considered non-motile, some strains have been found to exhibit motility through mechanisms such as twitching and surface-associated motility^[4]. The spread of virulent, antibiotic-resistant *Acinetobacter baumannii* is a global public health concern. Despite food animals being key in transmitting pathogens to humans, few studies report *A. baumannii* in them. Hence, the present study was designed to document the occurrence, antimicrobial resistance pattern, and virulence potential of *Acinetobacter baumannii* isolated from meat samples (chicken, mutton, and beef) marketed in retail outlets in Chennai, India.

MATERIALS AND METHODS

Study Samples

A total of 195 fresh raw meat samples (65 each of chicken, mutton, and beef), weighing approximately 20 g each, were aseptically collected in sterile polythene zip-lock pouches from retail outlets in Chennai, India, and transported to the laboratory on ice packs.

Reference Strains

The reference strain used in this study was *Acinetobacter baumannii* (MCC 2366) obtained from the National Centre for Microbial Resource, Pune, India.

Isolation Of *Acinetobacter Baumannii*

Five grams of each meat sample was enriched in 25 mL of sterile Brain Heart Infusion Broth (BHIB) and incubated overnight at 37°C. Enriched samples were streaked onto Leeds *Acinetobacter* Agar (HiMedia, India), and suspected colonies were identified using Gram staining, catalase, oxidase, and citrate utilization tests following standard laboratory protocols.

Confirmation by PCR

The colonies identified based on biochemical characterization were confirmed for *A. baumannii* by PCR targeting species-specific *A.b hyp* gene. DNA extraction of the isolates was done using the DNA extraction kit (Qiagen, USA). The reactions were carried out in a Thermal Cycler (DLAB TC1000S, China) with primer pair and conditions as shown in Table I. The amplifications were carried out according to Hamouda et al.^[5] The PCR amplicons were analysed by Agarose Gel electrophoresis carried out on 1.2% agarose gel, and the products with a fragment amplification of 545bp were considered positive.

Table I. Primer used in the study for identification of *Acinetobacter baumannii* and cycling conditions

Gene	Primer sequence	Condition	Cycles	Product Size(bp)	
<i>A.b_hyp</i>	<i>A.b_hyp</i> CGTCGGTCCGATCCGTGTAT <i>A.b_hyp</i> AAGTAAAGTGGCAGGCGCTT	Initial denaturation	95°C - 5 min	30 cycles	545
		Denaturation	94°C - 30 sec		
		Annealing	55°C - 30 sec		
		Extension	72°C - 45 sec		
		Final Extension	72°C - 2 min		

Antimicrobial Susceptibility Patterns

The antimicrobial resistance pattern of each *Acinetobacter baumannii* isolate was studied by the Kirby-Bauer disc diffusion method. A single colony of each was propagated in BHIB at 37°C for 6-8 hours, and a swab suspension adjusted to 0.5 McFarland standard was spread on the surface of Mueller- Hinton agar plate. (HiMedia, India). Octadisc (HiMedia, India) containing chloramphenicol (25 µg), ampicillin (25µg), tetracycline (25µg), gentamicin (30µg), co-trimoxazole (25µg), ceftriaxone (30µg), cefuroxime (30µg), ciprofloxacin (10µg) was gently pressed onto the inoculated plates and incubated at 37°C for 20 hours. The susceptibility was measured according to CLSI guidelines [6].

Motility Assay

The surface motility of the isolates was assessed on Luria Bertani soft agar (0.6%) (HiMedia, India). Surface motility was assessed by placing 10 µl of the test culture at the centre of the agar plate. The diameter of the drop was measured. The diameters were measured again after the overnight incubation at 37°C according to the criteria of Aliramezani et al. [3]

Haemolytic Assay

Haemolytic ability of the isolates was assessed on 5% Sheep Blood Agar supplemented with 5% sterile, defibrinated sheep blood and inoculated in a grid pattern [7]. After solidification, a drop from the test culture was placed on the surface of the agar in a grid pattern. The plates were incubated at 37°C for 48 hrs and were observed for the type of haemolysis.

Biofilm Formation Assay

The biofilm formation ability of the isolates was assessed by the Microplate Assay [8]. 200µl of the bacterial suspension was added into individual wells of a 96-well plate in duplicates and then incubated for 48 hours at 37°C. After incubation, the plates were washed with distilled water, stained with 1% crystal violet for 20 min at room temperature, and washed again. The bound dye was solubilized with 95% ethanol, and absorbance was measured at 570 nm using a microtiter plate reader.

Antibiotic and Virulence Factors Genes Detection

The genes encoding for antimicrobial resistance and virulence factors used in this study and the PCR conditions used for the genes are summarized in Table II. PCR products were analyzed by agarose gel electrophoresis (Medox, Chennai) at 100 V for approximately 1 h, visualized using a Gel Doc™ XR+ system (Bio-Rad), and sequenced by Eurofins Genomics India Pvt. Ltd., Bangalore.

Table II. Primers used in for virulence and antimicrobial resistance genes detection.

Target Gene	Primer sequence (5' -3')	Reaction condition		Cycles	Product Size(bp)
		PCR Step	Temp. & Time		
<i>bap</i>	<i>bapF</i> - TGCTGACAGTGACGTAGAACCACA <i>bapR</i> - TGCAACTAGTGGAATAGCAGCCCA	Initial denaturation	94°C - 5 min	35 cycles	184
		Denaturation	94°C - 1 min		
		Annealing	60°C - 30 sec		
		Extension	72°C - 45 sec		
		Final Extension	72°C - 5 min		
<i>bla_{PER}</i> - 1	<i>bla_{PER}</i> -1 F GCAACTGCTGCAATACTCGG <i>bla_{PER}</i> -1 R ATGTGCGACCACAGTACCAG	Initial denaturation	94°C - 5 min	35 cycles	900
		Denaturation	94°C - 1 min		
		Annealing	60°C - 30 sec		
		Extension	72°C - 45 sec		
		Final Extension	72°C - 5 min		
<i>csu E</i>	<i>csuE-F</i> - CATCTTCTATTTTCGGTCCC <i>csuE-R</i> - CGGTCTGAGCATTGGTAA	Initial denaturation	94°C - 5 min	35 cycles	168
		Denaturation	94°C - 45 sec		
		Annealing	60°C - 1 min		
		Extension	72°C - 1 min		
		Final Extension	72°C - 5 min		
<i>omp-A</i>	<i>omp-A-F</i> - CCAGTGTTATCTGTGTGACC <i>omp-A-R</i> - GTTAAAGGCGACGTAGACG	Initial denaturation	94°C - 5 min		578
		Denaturation	94°C - 1 min		

			Annealing	59°C - 1 min	35 cycles	
			Extension	72°C - 1 min		
			Final Extension	72°C - 10 min		
<i>pgaB</i>	<i>pgaB</i> – F - ATGCATCTGGGCTTGCATCT <i>pgaB</i> – R GTGACAGAGCAGGCAAAAGC	-	Initial denaturation	94°C - 5 min		190
			Denaturation	94°C - 45 sec	35 cycles	
			Annealing	64°C - 1 min		
			Extension	72°C - 1 min		
			Final Extension	72°C - 5 min		
OXA-51-like	OXA-51-like TAATGCTTTGATCGGCCTTG OXA-51-like TGGATTGCACTTCATCTTGG	F- R-	Initial denaturation	94°C - 3 min	35 cycles	353
			Denaturation	94°C - 45 sec		
			Annealing	57°C - 45 sec		
			Extension	72°C - 1 min		
			Final Extension	72°C - 5 min		
All <i>bla</i> _{TEM} genes	<i>bla</i> _{TEM} CATTTCGTGTCGCCCTTATTC <i>bla</i> _{TEM} CGTTCATCCATAGTTGCCTGAC	F- R-	Initial denaturation	95°C - 5 min	35 cycles	800
			Denaturation	94°C - 45 sec		
			Annealing	57°C - 45 sec		
			Extension	72°C - 1 min		
			Final Extension	72°C - 10 min		
<i>Int 1</i>	<i>Int1</i> F – CAGTGGACATAAGCCTGTTC <i>Int1</i> R- CCCGAGGCATAGACTGTA		Initial denaturation	94°C - 5 min	30 cycles	160
			Denaturation	94°C - 45 sec		
			Annealing	57°C - 45 sec		
			Extension	72°C - 1 min		
			Final Extension	72°C - 5 min		

RESULTS & DISCUSSION

Genomic Occurrence of *A.baumannii* in Meat

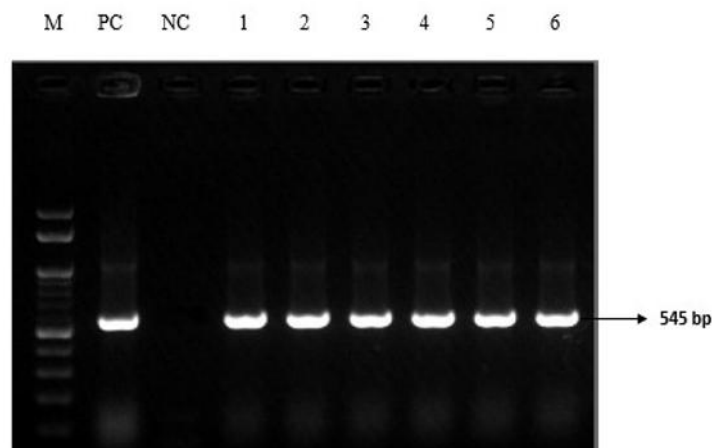
From a total of 195 samples, *Acinetobacter baumannii* was isolated from 4 beef samples (6.15%), 2 chicken samples (3.07%) and none from the mutton samples. (Table III) The result obtained is in accordance with the findings of 0.7% [9] and 4% [10] in Portugal, 8.2% [11] in Egypt, and 6.6% [12] in Haryana, India. On the contrary, Askari et al. [2] reported a high positivity percentage from sheep and goat meat of 41.12% and 11%, respectively from Shahrekord, Iran, and Lupo et al. [13] reported 25% of meat samples from Switzerland. The possible reasons for the variation in the occurrence of *Acinetobacter baumannii* from meat samples may be attributed to various factors like sampling method, geographical variation, protocols adapted for the isolation and identification of the isolates, PCR gene target, usage of contaminated water in slaughterhouses, and reduced sanitary conditions in the maintenance of equipment like knives.

Table III. Occurrence of *A.baumannii*

S.No	Sample	Presumptive colonies	Cultural and biochemical positive	PCR positive	Occurrence (%)
1	Chicken meat	8	2	2	3.07
2	Mutton	2	0	0	0
3	Beef	12	4	4	6.15
	TOTAL	22	6	6	3.07

Confirmation by PCR and Phylogenetic analysis of the Isolates

PCR targeting the *A.b_hyp* gene revealed that 3.07 percent of samples showed an appropriate product size of 545 bp (Fig.1). The sequences of the purified PCR products were annotated using DNA base sequence assembler version 5.15 and designated as Ab VPH 1, Ab VPH 2, Ab VPH 3 and Ab VPH 4. The sequences were analysed on BLAST by NCBI, and the per cent homology was identified ranging from 90 – 98%. Very closely related sequences were aligned and used in Phylogenfr programme for constructing a phylogenetic tree (Fig.2)



M: DNA marker (100bp); PC- Positive control; NC – Negative control; 1-6: Samples

Fig.1. Agarose gel electrophoresis (1.2%) of PCR product of *A_b.hyp* gene from *A.baumannii* isolated from meat samples from retail outlets in Chennai, India.

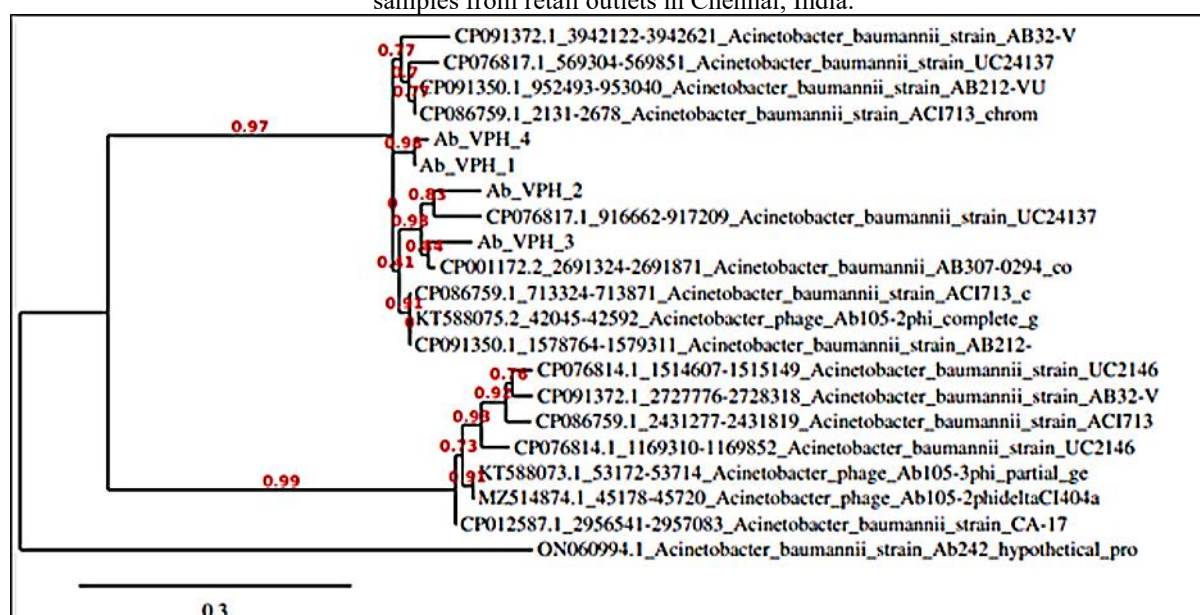


Fig.2. Phylogenetic tree of the isolates showing the relatedness of all the isolates. The isolates from the current study are denoted as Ab VPH 1, Ab VPH 2, Ab VPH 3 and Ab VPH 4.

The phylogenetic tree revealed two major clade with 0.99% and 0.97% variations. All our isolates were grouped in the second clade with 0.97% variation from the reference sequence (Accession No.ON060994.1). Further, the second clade had three major sub clades and 4 minor sub clades. However, our sequences viz. Ab_VPH 2 and Ab_VPH 3 were more closely related to *Acinetobacter baumannii* strain UC24137 (Accession No. CP076817.1) and *Acinetobacter baumannii* strain AB3070249_co (Accession No. CP001172.2) with a variation of 0.83% and 0.84% variability, respectively.

Other two strains viz. Ab VPH4 and Ab VPH 1 were very closely related, but a variation of 0.98% was detected. The strain, most related to the Ab_VPH 2 query sequence according to the database, revealed a hospital-based origin where the isolates were obtained from clinical samples collected in Chilean hospitals [14] whereas the Ab_VPH 3 query sequence was shown to be most related to the isolate obtained from the blood of a hospitalized patient in New York [15]. Phylogenetic analysis clearly indicated that our isolates matched the isolates of clinical origin, which is an important clue that documents the adaptation of the isolates to various systems, which needs to be monitored for the occurrence, considering the antimicrobial nature of this pathogen.

Antimicrobial Susceptibility

Confirmed *A. baumannii* isolates were subjected to phenotypic characterisation by the disc diffusion method. The antimicrobial susceptibility pattern is shown in Table IV. Results revealed that the 100% isolates showed sensitivity to gentamicin, co-trimoxazole, and tetracycline, followed by 83.3% sensitivity to ciprofloxacin in contrast to the 100% resistance shown to chloramphenicol and cefuroxime followed by ampicillin (50%) and ceftriaxone (33%).

High resistance was found to ceftriaxone at 89%, whereas 55.6% of isolates were sensitive to sulfamethoxazole/trimethoprim (co-trimoxazole) while showing no resistance to ciprofloxacin, which is in accordance with the result obtained in the current study [11]. A study [16] reported that the highest resistance was found against ampicillin (100%) in isolates obtained from meat and fish, whereas Carvalho et al. [10] found that 57.1% of the

isolates were sensitive to tetracycline. A study on isolates collected from animal-related samples in major Scottish abattoirs revealed that all 16 isolates were sensitive to gentamicin and tetracycline and completely resistant to chloramphenicol [17]. In the current study, our isolates largely showed a sensitivity pattern to the antibiotics tested. Some opined that *Acinetobacter baumannii* isolates from meat samples were generally susceptible to clinically relevant antibiotics [13]. Further, a study in the USA on antimicrobial resistance in *A. baumannii* strains obtained from various clinical strains collected from different countries clearly indicated that regional differences in antimicrobial resistance patterns are a common phenomenon between isolates in various countries [18]

Table IV. Antimicrobial resistance pattern for the obtained isolates of *A.baumannii* (disc diffusion method)

Sampl es	Chlorampe nicol	Ampicil lin	Tetracycl ine	Gentami cin	Co- Trimoxaz ole	Ceftriax one	Ciprofloxa cin	Cefuroxi me
C17	R	I	S	S	S	I	S	R
C25	R	R	S	S	S	S	S	R
B6	R	I	S	S	S	I	S	R
B29	R	I	S	S	S	I	S	R
B32	R	R	S	S	S	R	S	R
B50	R	R	S	S	S	R	I	R
Total	R = 6 (100%)	R = 3 (50%)	R = Nil	R = Nil	R = Nil	R = 2 (33%)	R = Nil	R= 6 (100%)
	I = Nil	I = 3 (50%)	I = Nil	I = Nil	I = Nil	I = 3 (50%)	I = 1 (16.67%)	I= Nil
	S = Nil	S = Nil	S = 6 (100%)	S = 6 (100%)	S = 6 (100%)	S = 1 (16.67%)	S = 5 (83.3%)	S= Nil

Motility Assay

Quantitative assessment of motility of the confirmed isolates revealed that 83.33% (5/6) showcased motility in the assay. One (16.67%) isolate showed high motility, 4 isolates (66.67%) showed intermediate motility and 1 (16.67%) showed negative motility (Fig.3).

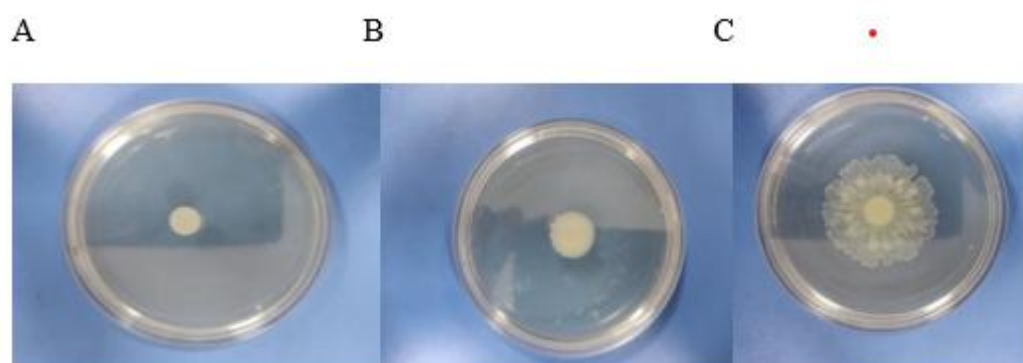


Fig.3. Motility assay for *A.baumannii* by soft agar method (a) Isolate showing negative motility (b) Isolate showing intermediate motility (c) Isolate showing high motility

All the isolates obtained from beef samples were motile in nature. This is in agreement with a study that reported that 80% of the *A.baumannii* isolates (72/95) showed surface motility on LB soft agar plate [19]. On the other hand, incidences of lower motility rates have been reported where only 35% of the isolates were found to be motile after 24 hours of incubation [3] and from a study where 27% clinical isolates showed evidence of surface motility from isolates of *A.baumannii* collected from a hospital in Kentucky [4]. In another study, isolates obtained from a hospital in Spain reported only 3 per cent of the isolates to be motile [20]. This is in complete contrast to the current study and their own previous study, where isolates from Lebanon showed motility. Motility patterns are highly variable among *A. baumannii* and the same is evinced in our study. As previously reported, environment-dependent variation in phenotypic motility makes motility a critical factor for understanding isolate behaviour and adaptation.

Haemolytic Assay

Four isolates (66.67%) showed clear zones of haemolysis around the colony formed after 48 hours of incubation (β - haemolysis) and remaining 2 (33.34%) isolates showed no presence of haemolytic activity (γ -haemolysis).(Fig.4) While both the isolates from chicken samples showed haemolytic activity (100%), only 2 of the isolates from beef samples showed haemolytic activity (50%).

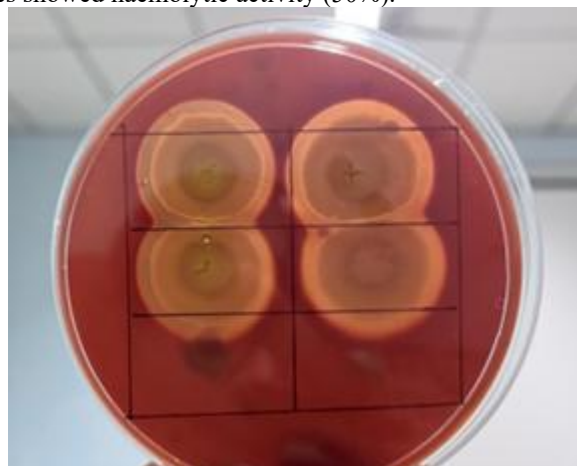


Fig.4. Haemolytic assay for *A.baumannii*. 4 isolates showed haemolysis and the last two colonies showed the absence of haemolytic activity

The haemolytic activity shown by the isolates in the present study is in agreement with the activity recorded in a study where 46.7% of the total *A. baumannii* isolates showed α -hemolysis, while one isolate showed β -hemolysis [19], and another study done by the same author described the presence of haemolytic activity in 54.2% of the total 59 isolates from hospital settings in Spain. However, in another study [7], all types of haemolytic activity were observed in the genus among different strains. In two extremely contradicting studies, it was found that no haemolytic activity was shown by any *A.baumannii* clinical strains obtained from Kentucky [4] whereas all the isolates from another study of clinical isolates of *A. baumannii* showed hemolytic ability, albeit having different intensity across strains [21]. This sheds light on the inconsistent presence of hemolytic activity in the strains obtained from various environments with varying intensities. Nevertheless, the presence of haemolytic activity, irrespective of its intensity, raises concerns about the virulence potential of an organism traditionally considered non-haemolytic.

Biofilm Formation Assay

The biofilm formation ability showed that a total of 4 isolates out of 6 *A.baumannii* isolates (66.67%) exhibited strong biofilm formation, whereas 2 isolates (33.34%) showed moderate biofilm formation. One (50%) and 3 (75%) of the isolates were strong biofilm producers from chicken samples and beef samples, respectively. (Table V)

The result obtained is in close conjunction with a study, where 58% of the isolates were strong biofilm formers and 42% exhibited moderate biofilm-forming abilities [22]. Furthermore, 77 (85.6%) of the *A.baumannii* isolates formed strong biofilms, and ten (11.1%) formed weak biofilm in another study by Dahdouh et al. [19]. While it is known that the clinical *A.baumannii* isolates obtained are generally strong biofilm producers, it has been observed in Turkey that 5 isolates of *A.baumannii* identified from various samples collected from the meat industry were strong (2/5) and moderate (3/5) biofilm formers [23]. Contamination of food processing surfaces and equipment by such isolates is a public health concern, as their transmission through processed foods, especially ready-to-eat products, may facilitate the spread of antimicrobial-resistant bacteria to humans.

Table.V. Biofilm formation of the isolates. OD₅₇₀ values of the isolates mentioned in mean $\pm$ S.E.

Sample No.	OD value	Interpretation
C17	1.933 $\pm$ 0.617	Strong
C25	0.403 $\pm$ 0.010	Moderate
B6	1.198 $\pm$ 0.178	Strong
B29	0.808 $\pm$ 0.063	Moderate
B32	0.997 $\pm$ 0.043	Strong
B50	1.127 $\pm$ 0.033	Strong

Distribution of Antimicrobial and Virulence Genes

Genotypic characterization for antimicrobial resistance was carried out by PCR targeting various antibiotic resistance genes namely OXA-51-like, OXA-23-like bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{KPC}, bla_{NDM}, qnrA, qnrB, tetA, tetB, and Int 1. The most common gene identified was the bla_{OXA-51-like} gene and Int-1 gene with an occurrence

rate of 100% in the isolates. This was followed by *bla*TEM gene which was present in 50% (3/6) isolates. (Fig.5-7) None of the isolates showed the presence of genes like *OXA-23-like*, *bla*SHV, *bla*CTX-M, *bla*KPC, and *bla*NDM. *tetA*, *tetB*, *qnrA*, and *qnrB*.

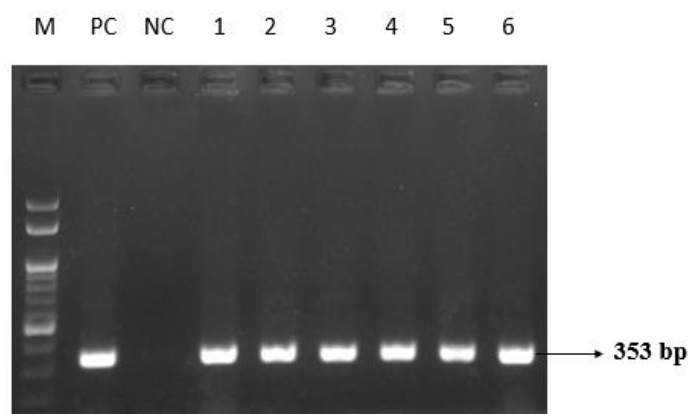


Fig.5. Agarose gel electrophoresis (1.2%) of PCR product of *bla*_{OXA-51} like gene
M: DNA marker (100bp); PC- Positive control; NC – Negative control; 1-6: Samples

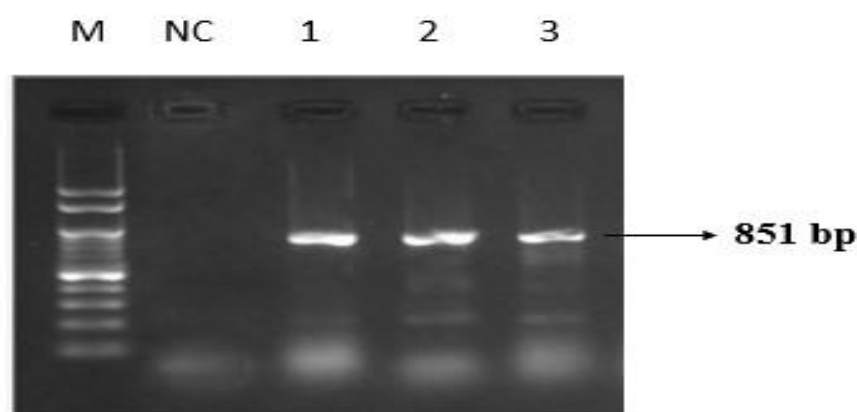


Fig.6. Agarose gel electrophoresis (1.2%) of PCR product of *bla*_{TEM} gene
M: DNA marker (100bp); NC – Negative control; 1-3: Samples

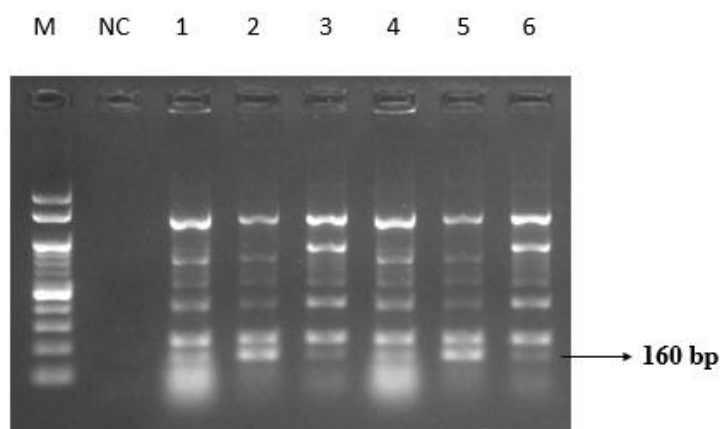


Fig.7. Agarose gel electrophoresis (1.2%) of PCR product of *Int 1* gene
M: DNA marker (100bp); NC – Negative control; 1-6: Samples

The most common subgroup in a study in the UK was *bla*_{OXA-51-like} genes (93% isolates) supporting the theory that they are unique to *A.baumannii* [24]. Another study [25] found that the *bla*_{OXA} subgroup-3 (100% of all isolates) including *bla*_{OXA-51} (98.9%) and *bla*_{TEM-92} (69.9%), were most commonly found. It was reported that most *A.baumannii* strains isolated from sheep meat samples in a study conducted from raw animal meat samples in Iran represented Integron Class I (96.07%) genes [2]. In contrast to the present study, another study detected *tetA* and *tetB* as one of the most common genes in the isolates in the study [26] with the phenotypic characterization of antimicrobial resistance of those isolates revealing the highest resistance to tetracycline. Thus, there is a varying trend seen in the presence of antimicrobial resistance genes in isolates throughout the world. Previous reports have highlighted that various factors like geographical location, antibiotic usage, farming conditions, feeding, and environment play a pivotal role in the occurrence of antibiotic resistance from a

study area.

Genotypic characterization of virulence factors found that 100% of the isolates showed the presence of the genes encoding for virulence factors except for *bla_{PER-1}* (66.6%). (Fig. 8 – 12) The distribution of the virulence genes and the antimicrobial genes among the isolates have been represented in Fig.13.

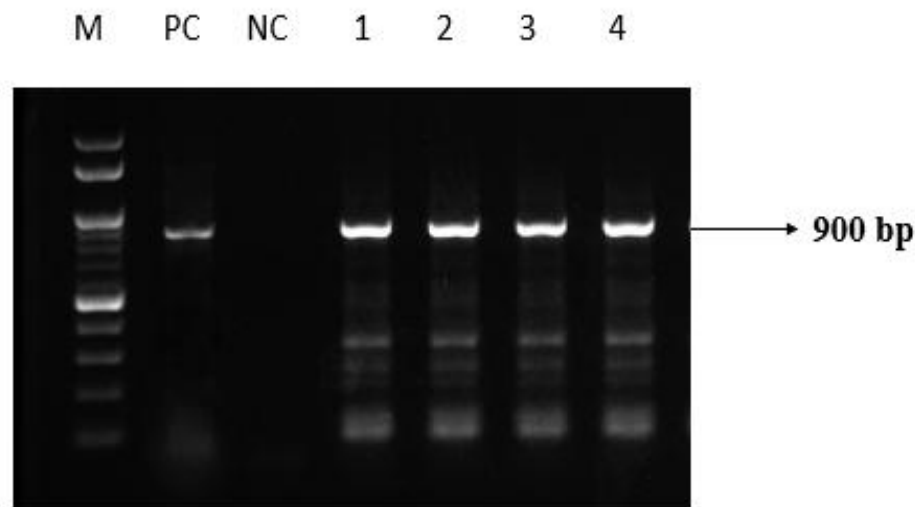


Fig. 8. Agarose Gel Electrophoresis (1.2%) product for *bla_{PER-1}* gene
M: DNA marker (100bp); PC- Positive control; NC – Negative control; 1-6: Samples

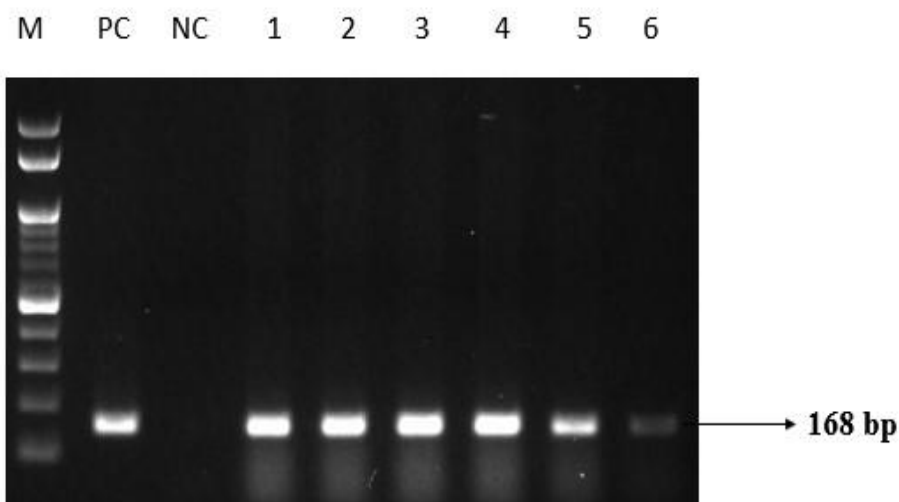


Fig.9. Agarose Gel Electrophoresis (1.2%) product for *csuE* gene
M: DNA marker (100bp); PC- Positive control; NC – Negative control; 1-6: Samples

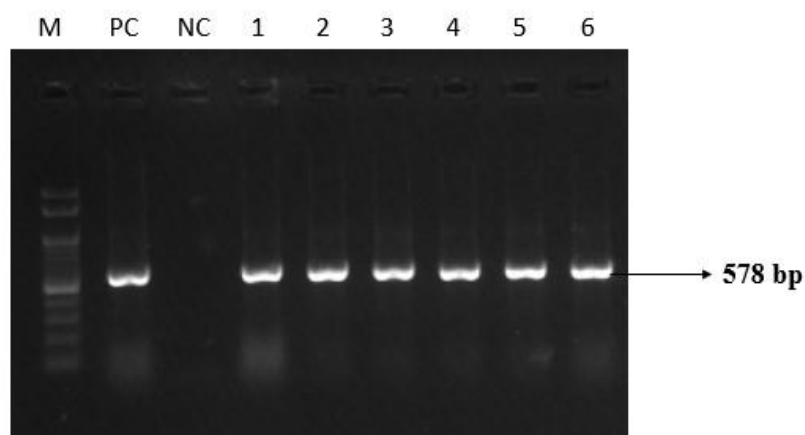


Fig.10. Agarose Gel Electrophoresis (1.2%) product for *OmpA* gene
M: DNA marker (100bp); PC- Positive control; NC – Negative control; 1-6: Samples.

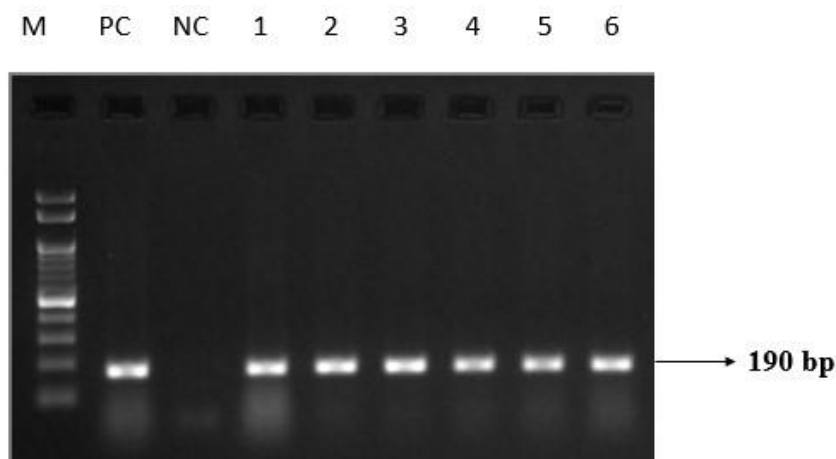


Fig. 11. Agarose Gel Electrophoresis (1.2%) product for *pgaB* gene
M: DNA marker (100bp); PC- Positive control; NC – Negative control; 1-6: Samples

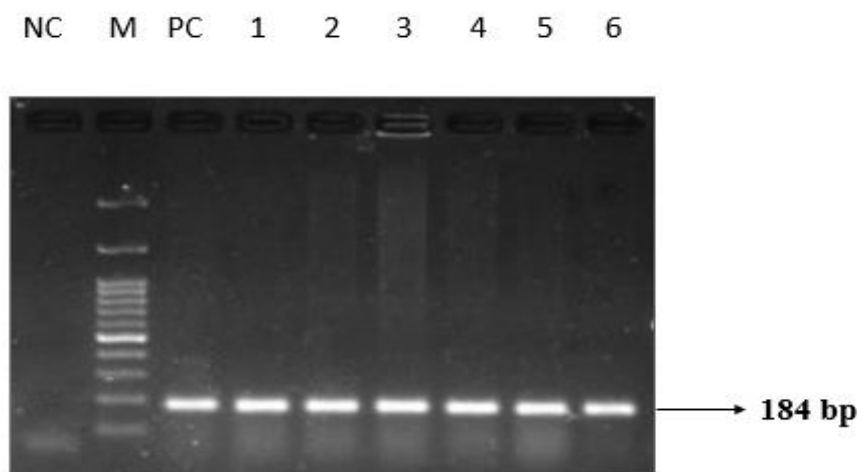


Fig.12. Agarose Gel Electrophoresis (1.2%) product for *bap* gene
M: DNA marker (100bp); PC- Positive control; NC – Negative control; 1-6: Samples

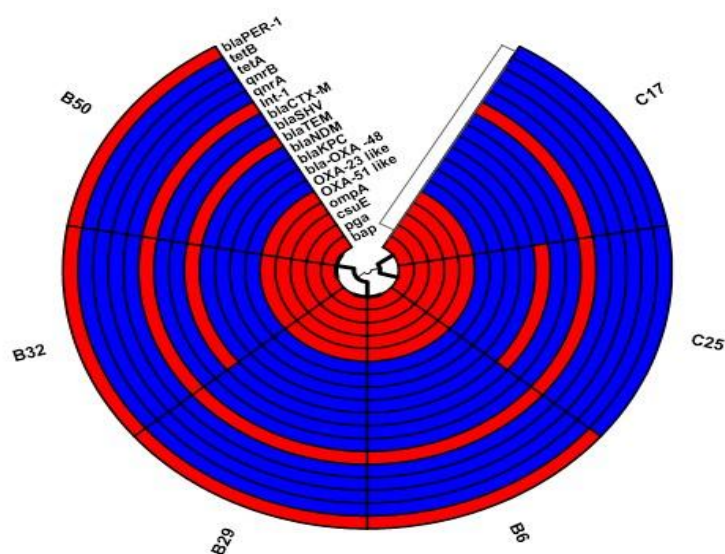


Fig.13. A Polar Heat Map generated from the data showing the distribution of different virulence and antimicrobial resistance genes in the isolates obtained. The Heat Map has shown the presence of 2 clusters (B50 and B32) and (B29 and B6) whereas both the isolates from chicken samples are excluded from the clusters.

The results are in conjunction with previous reports^[22] where presence of *csuE* gene was found in 100% of isolates, followed by *pgaB* (98%) and *OmpA* (81%). In another study, the frequencies of *OmpA*, *bla_{PER-1}*, and *bap* genes in clinical and environmental isolates were 100%, 53.3%, 82.7%, and 100%, 37.5%, and 84.4%, respectively from a hospital environment in Iran ^[27]. In a study on *A.baumannii* from hospitalized patients in intensive care units (ICUs) from Iran, it was reported that *OmpA* and *csuE* genes were found in all isolates, while *bap* and *bla_{PER-1}* were found in 43(66%) and 42(64%) of the isolates with strong and moderate biofilm activities [28]. The reduced frequency of the *bla_{PER-1}* gene observed in the current study is consistent with previous reports, including a study from Taiwan ^[29], where *bla_{PER-1}* had the lowest prevalence (38.3%) among the biofilm-related genes detected in 154 isolates, and another study ^[22], which reported an even lower prevalence of only 2% among the isolates. However, in this study, the presence of these genes in the obtained isolates is very well reflected in its exhibition of phenotypic virulent abilities.

The results of the current study indicate the presence of *A.baumannii* in meat samples from retail markets in Chennai, India. It should be noted that these isolates were obtained from meats for human consumption, linking the possible role of meats from the study area in the transmission of this highly virulent pathogen to humans via the food chain, thus calling into question food safety regulations. Therefore, several measures, such as quality control, proper monitoring, and hygiene maintenance, should be adopted to reduce the incidence of *A. baumannii* contamination in meat.

AUTHORS' CONTRIBUTION

Panicker Nidhi Sudhakaran¹: Carried out the experiments, data collection, and drafted the original manuscript. **Porteen Kannan^{2*} (corresponding author):** Conceived the study, provided supervision and guidance and revised the manuscript critically, **Sureshkannan Sundaram³:** Performed data analysis and evaluation, validated the results, and contributed to the interpretation of results, **Rajalakshmi Shanmugham⁴:** Supported the experimental work, provided critical input and assisted with data-curation, **Wilfred Ruban⁵:** Reviewed and edited the manuscript and contributed to refinement of the discussion and conclusions, **J Karthikeyan⁶:** Contributed to the sample collection and preparation of the manuscript, **Takekar Gayatri Abhijit⁷:** Contributed to preparation of the manuscript and data assessment.

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Declaration of conflicting interests

There were no conflicts of interest disclosed by the author(s) in connection with the research, authorship, and/or publication of this article.

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