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Original article

Acinetobacter baumannii as a community foodborne pathogen: Peptide mass fingerprinting analysis, genotypic of biofilm formation and phenotypic pattern of antimicrobial resistance

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ABSTRACT

Acinetobacter baumannii (*A. baumannii*) is one of the most common Gram-negative pathogens that represent a major threat to human life. Because the prevalence of Multidrug-resistant biofilm-forming *A. baumannii* is increasing all over the world, this may lead to outbreaks of hospital infections. Nonetheless, the role of raw meat as a reservoir for *A. baumannii* remains unclear. Here our research was aimed to exhibit the frequency, precise identification, and genotyping of biofilm-related genes as well as antimicrobial resistance of *A. baumannii* isolates of raw meat specimens. Fifty-five *A. baumannii* strains were recovered from 220 specimens of different animal meat and then identified by Peptide Mass Fingerprinting Technique (PMFT). All identified isolates were genotyped by the qPCR method for the existence of biofilm-related genes (*ompA, bap, blaPER-1, csuE, csgA, and fimH*). In addition, the antimicrobial resistance against *A. baumannii* was detected by the Kirby-Bauer method. Based on our findings, the frequency rate of 55 *A. baumannii* isolates was 46.55%, 32.50%, 15.00%, and 9.68% of sheep, chicken, cow, and camel raw meat samples, respectively. The PMFT was able to identify all strains by 100%. the percentages of *csuE*, *ompA*, bla_{PER}*-1, bap,* and *csgA* genes in biofilm and non-biofilm producer *A. baumannii* were 72.73%, 60%, 58.2%, 52.74%, and 25.45%, respectively. In contrast, the *fimH* was not detected in all non-biofilm and biofilm producer strains. The *ompA*, *bap, blaPER-1, csgA* were detected only in biofilm-producing *A. baumannii* isolates. The maximum degree of resistance was observed against amoxicillin/clavulanic acid (89.10%), gentamicin (74.55%), tetracycline (72.73%), ampicillin (65.45%), and tobramycin (52.73%). In conclusion, our investigation demonstrated the high incidence of multi-drug resistant *A. baumannii* in raw meat samples, with a high existence of biofilm-related virulence genes of *ompA, bap, blaPER-1, csgA*. Therefore, it has become necessary to take the control measures to limit the development of *A. baumannii*.

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1. Introduction

Increasing consumption of fresh, undercooked food is not only considered to be the most important leading cause of foodborne diseases worldwide (Damaceno et al., 2015; Elbehiry et al., 2017), but it is also linked to numerous eruptions of bacterial

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pathogens (Safarpoor-Dehkordi et al., 2017; Askari et al., 2020). Although meat is considered an important source of protein, it likely to decay or go bad quickly for the reason that it delivers a favorable environment for the development of numerous microorganisms (Bantawa et al., 2018). Lack of interest in healthy meat throughout the processing leads to contamination with many harmful bacteria including Acinetobacter species (Zhang et al., 2014; Askari et al., 2020).

Acinetobacter species are saprotroph, omnipresent and have developed as an imperative hospitalized bacterium as a result of its capability for the existence in a wide range in the hospital surroundings (Tavakol et al., 2018). *Acinetobacter baumannii* (*A. baumannii*), is a Gram-negative coccobacillus and considered one of the recently emerged Acinetobacter species in many parts of the world (Tavakol et al., 2018). *A. baumannii* represents one of the most important nosocomial microorganisms, particularly in intensive care units (ICUs), which lead to several illnesses for example pneumonia, septicemia, urinary tract infection, nosocomial meningitis, wound infection, and skin infection (Askari et al., 2019). Captivatingly, it can be recognized in various foodstuffs as fruits, raw vegetables, raw milk, and milk products (Almasaudi, 2018).

To date, insufficient researches of infections caused by *A. baumannii* in animals, and only a few investigations have been described for such cases. Carvalheira et al. (2016) and Anane et al. (2019) revealed that the diseases caused by *A. baumannii* strains recovered from extra-hospital contribute to the hospitalized illnesses. This proposes that the source of this bacterium comes from outside the hospital and consequently the importance of ecological isolates in the incidence, distribution, and possible control of *A. baumannii* is of obvious anxiety extending throughout the world. Up to the present time, there is no clear data regarding the method of the entrance of *A. baumannii* into the health institutions (Anane et al., 2019).

Although, various practices have been established to identify various bacterial pathogens, comprising culturing and biochemical techniques, which permit the detection of numerous types of microorganisms. Nonetheless, some of them aren't identified definitely with these techniques. The genotypic method also represents an accurate method for identifying various microorganisms (Chen et al., 2014; Elbehiry et al., 2019), but unfortunately, they aren't extensively applied as a result of their higher cost, labor-intensive, and time-consuming. Therefore, using rapid and accurate technologies has recently become a very important challenge, especially with microorganisms that are difficult to isolate or that need a long time to identify.

From this perspective, our investigation was aimed to use Peptide Mass Fingerprinting (PMF) as modern technology for the rapid characterization of *A. baumannii*. This practice could be an influential technique in the analysis of *A. baumannii* since it lets not only the identification of microorganisms on the genus level but also on the species level. This technology has developed as a major technology for the recognition of various microorganisms in clinical and veterinary microbiology laboratories and has developed detection of *A. baumannii* complex organisms (Espinal et al., 2012; Leung et al., 2019). Its mechanism of action based mainly on a mass spectrum of bacterial proteins for pathogen detection compared with the stored mass spectrum from well-known sources (Elbehiry et al., 2017).

Current and previous researches stated that the capability of *A. baumannii* to yield biofilm may be related to numerous virulence genes (Avila-Novoa et al., 2019). The biofilm-associated protein (bap) gene is considered one of the most important virulence genes in *A. baumannii* which plays a vital role in biofilm formation and intercellular adhesion (Fattahian et al., 2011; Aliramezani et al., 2016). In the previous studies, the existence of bla_{PER-1} gene has been also recognized to stimulate the A. baumannii to produce biofilm and intracellular attachment (Lee et al., 2008; Loehfelm et al., 2008; Brossard and Campagnari, 2012). Furthermore, the study conducted by Loehfelm et al. (2008) exhibited that the outer membrane protein A (OmpA) gene in A. baumannii demonstrates an incomplete role in the formation of biofilms on plastic surfaces.

Among the family of *Acinetobacter*, *Acinetobacter baumannii* has been considered one of the major hazards in hospitalized infections, as a result of their capability to acquire resistance to manifold antimicrobial agents and to persist in hospital settings. Nevertheless, virulent *Acinetobacter* species have also been established in food-producing animals, which could consider a reservoir and source of infection for humans (Marí-Almirall et al., 2019).

A. baumannii strains exhibited more resistant patterns than other Acinetobacter species and frequently show a multi-drug resistant (MDR) phenotype. Subsequently, throughout the last 3 decades, *A. baumannii* strains have revealed tolerance against recently established antibiotics (Askari et al., 2019). This fact has become predominant in certain hospitals worldwide and has been recognized as a complex hospitalized bacteria (Dahiru and Enabulele, 2015). Different types of antibiotics are necessary for the treatment of human and animal infections (Guardabassi et al., 2004).

According to the degree of antibiotic resistance, *Acinetobacter* spp. illustrated three various terms: multidrug-resistant (MDR), extensive drug-resistant (XDR), and pan-drug-resistant (PDR) (Askari et al., 2019). Increased mortality, infection rate, and MDR caused by *A. baumannii*, so this pathogen is considered to be very dangerous to human health at the present time (Amorim and Nascimento, 2017). However, one of the common causes why *A. baumannii* draws attention in the hospitals is its unusual aptitude to acquire and accumulate genetic determinants that confer resistance to various antibiotics, leading to infections caused by strains showing the MDR phenotype (Novovicet al., 2015).

Based on the reports submitted by the CDC, *A. baumannii* is associated with approximately 7300 infections and 500 deaths per year and it is considered as a protuberant bacterium in hospitalized-acquired infections. A list of 56 antimicrobial-resistant pathogens was established by WHO and presented the *A. baumannii* its maximum significance level of danger, mostly as a result of the deficiency of treatment opportunities existing at this time. From this perspective, both the CDC and WHO have suggested that novel diagnostics and therapeutics are considered an urgent matter to face the world-wide danger of antibiotic-resistant bacteria, comprising *A. baumannii*.

Because of investigations concerning the correlation of *A. baumannii* strains with infected food are slightly restricted, therefore, the current research was achieved to scan the frequency, precise identification, and genotyping of biofilm-related genes as well as the phenotypic configuration of antimicrobial resistance against *A. baumannii* strains of various raw meat.

2. Materials and methods

2.1. Samples collection

A total of 220 raw meat specimens including dromedary camels (n = 66) cows (n = 60), sheep (n = 58), chicken (n = 40) meat specimens were arbitrarily collected from the slaughterhouses and shopping centers of various parts of the Buraydah city, Al-Qassim Province, Saudi Arabia from January to May 2019. A total of 30 g of meat specimen was collected aseptically from each animal. All specimens were directly transported under hygienic measures in a cooler with ice packs to the Microbiology laboratory. All meat specimens displayed normal physical properties comprising odor, color, and texture.

2.2. Bacterial isolation

Fifteen grams of meat sample was mixed in 85 mL of Lysogeny broth (Difco, BD-Canada) for 30 s using The Fisher Scientific™ 850 Homogenizer (Thermo Fisher Scientific, USA) and then incubated overnight at 37 °C with shaking. According to the protocol described by Tavakol et al. (2018), 10 μl from this incubated culture was inoculated onto selective Chromogenic medium (ChromID ESBL agar, bioMérieux, France), which can't inhibit the growth of *A. baumannii*, and was incubated at 37 °C for one successive day. All suspected colonies which show white color were moved onto blood agar plates (Sigma Aldrich, USA) and MacConkey agar then kept at 37 °C for 24 h.

After cultivation, the oxidase, urease, citrate, malonate consumption, motility and indole tests were carried out as biochemical analysis to identify *A. baumannii*. Colonies that displayed cloudy and creamy color on blood agar and non-lactose fermenter on MacConkey agar were sub-cultured again on the MacConkey agar and incubated for at 37 °C for 24 h to obtain pure colonies. According to the colony morphology, microscopic examination, and various biochemical analysis, the isolates were identified as *A. baumannii*. Typical cultures were preserved in a sterile buffered glycerine (20%) and CryoBank vials (COPAN Diagnostics Inc., California, United States) at −86 °C for further investigations.

2.3. Peptide mass fingerprinting technique (PMFT) for identification of A. baumannii

Based on the PMFT described by Bruker Daltonics (Bremen, Germany), ethanol-formic acid-acetonitrile extraction protocol (Barreiro et al., 2010) was applied for proteomic identification of different isolates of *A. baumannii* recovered from meat samples collected from various animal species. In brief, 300 μl of deionized water was transferred into an Eppendorf tube. After culturing of bacteria on MacConkey agar at 37 °C for 24 h, one single fresh colony was inoculated onto the tube and then carefully mixed. After that, 900 μl of absolute ethanol was added, mixed comprehensively, and then rotated at 13,000–15,000 rpm for two minutes (Centrifuge 5430, Eppendorf, USA). The supernatant was then discarded, and centrifugation was carried out again and all the residual ethanol was removed by carefully pipetting it off to waste without disturbing the pellet.

The absolute ethanol-pellet was then dried at room temperature for two minutes. Then, 80 μl of 70% formic acid was transferred to the pellet and diversified very well by pipetting and/or by vortexing. After that 80 μl of pure acetonitrile was added and carefully mixed, (We used the same percentage as formic acid, 70%). Then centrifugation was carried out for 2 min at maximum speed. After that 1 μl of supernatant was inoculated onto a MALDI target plate and was permitted it to dry at 23–25 °C. Finally, 1 μl of alpha-Cyano-4-hydroxycinnamic acid (HCCA) solution was overlaid onto the entire spot within 1 h and was left to dry at 23–25 °C. Running of all samples was carried out via Compass and Flex-Control software stored in the Microflex LT device.

2.4. Biofilm formation

The aptitude of *A. baumannii* to produce biofilm was detected via the microtitre plate technique as stated formerly by Toledo-Arana et al. (2001). Briefly, the isolates of *A. baumannii* were incubated at 37 °C for 24 h in trypticase soy broth (TSB) comprising 0.25% glucose. After removal of the free cells, biofilms were washed several times with a sterilized water-based salt solution and then stabilized with 150 mL of 99% (v/v) methanol (methyl alcohol), anhydrous 99.8% (Sigma-Aldrich, USA). At room temperature, the wells were then stained with crystal violet aqueous solution 1% (Sigma-Aldrich, USA) for 30 min. Ethanol/acetone 33% was then added to dissolve the crystal violet for 20 min and the optical density (OD) was estimated at 620 nm. Scoring of biofilm production was determined as non-biofilm former (OD_{620} < 0.275), weak biofilm former (0.275 ≤ OD 620 < 0.55), medium biofilm former (0.55 ≤ OD_{620} < 0.825) and strong biofilm former (0.825 ≤ OD620). Each test was carried out in duplicate and the average of OD was taken.

2.5. Determination of the extended-spectrum β-lactamases (ESBL) production

The prevalence of ESBL production in *A. baumannii* was detected applying the double-disc synergy test (DDST) which achieved based on the protocol designated formerly by Fallon and Young (2007).

2.6. Detection of metallo β-lactamases production (MBL)

A. baumannii strains that displayed resistance to imipenem by the agar disc diffusion test were used as screen test positives and were further examined for approval of MBL production. In brief, two discs of imipenem (10 μg) were located on a lawn plate of the isolates to be verified and 10 μl of 0.5 M EDTA (Sigma-Aldrich, USA) solution was added to one disc of imipenem. The imipenem discs alone and those with EDTA were compared after 24 h to determine the inhibition zone. The imipenem -EDTA disc with ≥7 mm was considered as a positive result (Saha et al., 2010).

2.7. Genomic identification of biofilm-related genes in A. baumannii using qPCR

2.7.1. DNA extraction

DNA extraction of *A. baumannii* strains was accomplished using Genesig Advanced Kit (Genesis, USA). The extraction procedure was implemented as stated by the manufacturer's approvals.

2.7.2. qPCR detection protocol

In the current investigation, we used qPCR System (Thermo Fisher Scientific, USA) for amplification of *16S-23S* ribosomal DNA and the biofilm-related genes were revealed in Table 1. Briefly, a 16 μl reaction volume comprising 10 μl of oasig or PrecisionPLUS 2X qPCR Master Mix, 1 μl *A. baumannii* primer/probe mix, 1 μl internal extraction control primer/probe mix, 1 μl target DNA, and 1 μl of purified water were applied. All reactions were run two times. The magnification protocol was achieved as enzyme activation at 95 °C for 2 min, denaturation at 95 °C for 10 s, data collection at 60 °C for 60 s, all was achieved for 50 amplification cycles. Amplification outcomes were stated by plotting Delta Rn (ΔRn).

2.8. Antibiotic susceptibility testing and multiple antibiotics resistance index (MARI)

The response of *A. baumannii* against 9 antimicrobial drugs was investigated on Müller-Hinton agar (Sigma-Aldrich, USA) using agar disc diffusion test (Kirby-Bauer method), as stated by the CLSI M100-S21 recommendations (Poirel and Nordmann, 2006). The following antibiotics discs (Mast Diagnostica GmbH, Germany) were used in our experiment: ampicillin (10 μg), piperacillin (100 μg), amikacin (30 μg), gentamicin (10 μg), tetracycline (30 μg) tobramycin (10 μg), amoxicillin-clavulanic acid (20 μg), ceftazidime (30 μg), cefepime (30 μg), imipenem (10 μg). In this investigation, we used *A. baumannii* ATCC 19606 as a control positive strain. The findings were explained as stated by the rules and regulations of CLSI (2017). The resistance of *A. baumannii* to

Table 1
Oligonucleotide sequences utilized for detection of biofilm-related virulence genes in *A. baumannii.*

Target gene	Primer sequences (5′-3′)	Base pair	Reference
ompA	GTTAAAGGCGACGTAGACG CCAGTGTTATCTGTGTGACC	578	Smani et al. (2014)
bap	ATGCCTGAGATACAAATTAT GTCAATCGTAAAGGTAACG	1449	Badmasti et al. (2015)
bla_{PER}-1	ATGAATGTCATTATAAAAGC AATTTGGGCTTAGGGCAGAA	925	Strateva et al. (2007)
csuE	CATCTTCTATTTCGGTCCC CGGTCTGAGCATTGGTAA	168	Azizi et al. (2016)
csgA	ACTCTGACTTGACTATTACC AGATGCAGTCTGGTCAAC	200	Darvishi (2016)
fimH	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	Johnson & Stell (2000)
16S–23SrDNA	CATTATCACGGTAATTAGTG AGAGCACTGTGCACTTAAG	208	Askari et al. (2019)

at least one agent in ≥ three groups of antimicrobial drugs was defined as multidrug resistance (MDR).

We analyzed and explained the results of MARI based on the method described previously by Krumperman (1983) as the proportion of antibiotic numbers to which the strains were tolerating (A) divided by the total antibiotics to which the strains were exposed (B), i.e: MARI = A/B. The MARI of *A. baumannii* recovered from the various meat samples was calculated and interpreted. *A. baumannii* that exhibited MARI more than 0.2 originates from a high-risk source of infection where numerous antimicrobial drugs are utilized. Whereas; the MARI value of less than or equal to 0.2 points to the isolate originated from sources where antimicrobial drugs are rarely or certainly not used (Krumperman, 1983).

2.9. Statistical analysis

Analysis of our results was applied by the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Frequency of A. baumannii strains in meat

A total of 220 raw meat specimens including dromedary camels (n = 62) cows (n = 60), sheep (n = 58), camel, chicken (n = 40) meat specimens were collected from the slaughterhouses of various localities of the Buraydah city; of these, *A. baumannii* were recovered from 6 (9.68%) camel's meat samples, 9 (15.00%) from cow's meat samples, 27 (46.55%) sheep's meat samples, and 13 (32.50%) from chicken's meat samples (Table 2). From these results, it is clear that the sheep meat is the most affected by *A. baumannii* followed by chicken. In contrast, the fewest isolates were isolated from camel meat.

3.2. PMFT for the identification of A. baumannii

In this investigation, 55 *A. baumannii* were analyzed using PMFT (Bruker Daltonik, GmbH, Bremen, Germany). Database version V.3.3.1.2 which permitted by the Food and Drug Administration (FDA) under Section 510(k) was used for the rapid identification of *A. baumannii* recovered from various meat samples. The mechanism of this technique was based on the comparison of the created Spectra from the tested samples with the spectra deposited in the software library. Based on our findings, 53/55 (96.36%) of *A. baumannii* were correctly identified by PMFT.

As shown in Table 3, PMFT was able to identify 34 out of 53 (64.15%) *A. baumannii* isolates, with a score value, ranging from 2.3 to 3. As well, 19 out of 53 (35.84%) *A. baumannii* were recognized, with a score value fluctuated from 2 to 2.299. On the other hand, at the genus level two *A. baumannii* isolates were only recognized with a score value ranged from 1.7 to 1.99. No misidentification was detected in the current investigation.

Table 2
Frequency of *A. baumannii* isolates in dromedary camel, cow, sheep and chicken raw meat samples.

Meat samples	No. of samples	No. of isolates	% of isolation
Camel meat	62	6	9.68%
Cow meat	60	9	15.00%
Sheep meat	58	27	46.55%
Chicken meat	40	13	32.5%
Total	**220**	**55**	**25%**

3.3. Principal component analysis created by PMFT

In addition, the principal component analysis (PCA) represents a supplementary calculated tool generated by the Compass software of MALDI Biotyper for analyzing data sets to illustrate the degree of resemblance and variety of various spectra of protein profile. Likewise, the PCA reduces the variances of a complex dataset as stated by the different algebraic assessments. A number of spectral proteins for *A. baumannii* strains were established in 3d- PCA as shown in Fig. 1A. Every spectrum was stated via dot and the various colors demonstrate the reflected group contribution in which every dot represented by one spectrum of the protein side view.

The cluster outlook of the 3d PCA illustrated that the majority of peaks for *A. baumannii* strains were strictly correlated and harmonized together (Fig. 1B). Regarding the PCA calculation sets, every single peak may perhaps develop loading values derived from the PCs calculation. In our investigation, every signal was identified with loading1, loading 2, and Loading 3 values resulted from the calculation of PC1, PC2, and PC3.

3.4. ESBL and MBL productions

Out of 55 isolates of *A. baumannii*, 17 (30.9%) were established to produce ESBL using the double-disc synergy test with one or more of the cephalosporins (class of β-lactam antibiotics) used. Moreover, 9 (16.37%) isolates were able to produce MBL via a combined disc diffusion technique.

3.5. Biofilm producing A. baumannii

Forty-one (74.54%) out of 55 *A. baumannii* isolates were able to form biofilm as follows; 10 (18.2%) isolates formed weak biofilm, 13 (23.64%) isolates formed moderate biofilm and 18 (32.73%) iso-

Table 3
Score values for 55 *A. baumannii* of various meat samples identified by PMFT.

Meat Samples	Total isolates	Score value of identified *A. baumannii*			
		2.300–3.000	**2.299–2.000**	**1.700–1.99**	**0.000–1.600**
Camel meat	6	4	2	0	0
Cow meat	9	5	4	0	0
Sheep meat	27	17	9	1	0
Chicken meat	13	8	4	1	0
Total	**55**	**34**	**19**	**2**	**0**

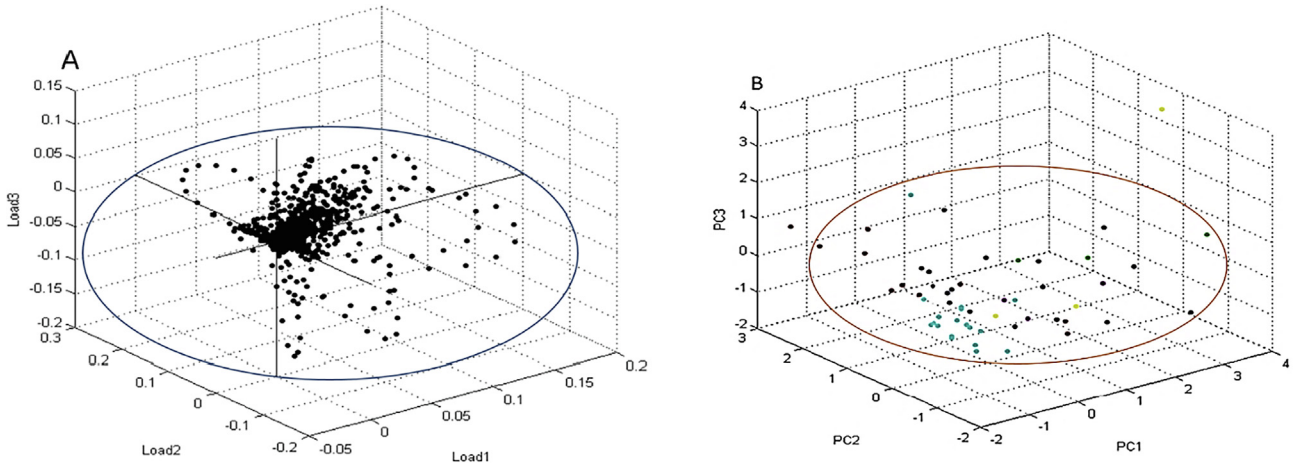

Fig. 1. The 3d loading image created by PCA illustrates numerous spectra for 55 *A. baumannii* meat strains **(A)** Each dot exhibited the force value of the peaks. The peaks were changed according to the loading value matching with the loading1, loading 2 and loading 3 model **(B)** The grouping of *A. baumannii* isolates in the first 3PC model (PC1, PC2, PC3).

Table 4
Scoring and numbers of *A. baumannii* biofilm producer.

Biofilm formation score	Non-producer		Biofilm producer					
			Weak producer		Moderate producer		Strong producer	
	No.	%	No.	%	No.	%	No.	%
	14	25.46	10	18.2	13	23.64	18	32.73
Total	**14 (25.46%)**		**41 (74.54%)**					

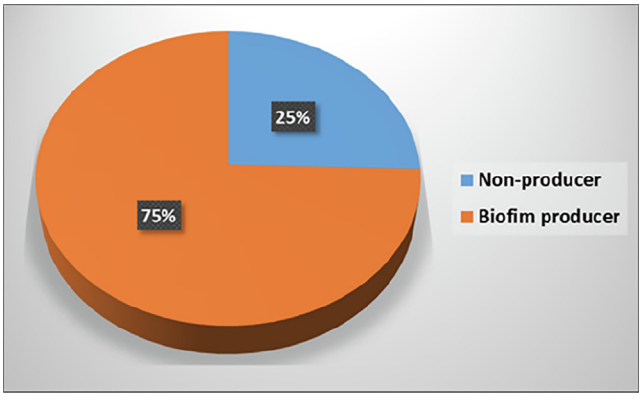

Fig. 2. Proportion of *A. baumannii* isolates that biofilm-producer and non-biofilm producer.

lates exhibited a strong capability to form a biofilm. While 14 (25.46%) *A. baumannii* strains were unable to form a biofilm (Table 4 & Fig. 2).

The vast majority of strains had the capability to produce biofilm. The mean ODs for all *A. baumannii* strains were 0.403 ± 0.02 3 (ranged from 0.018 to 0.988). The *A. baumannii* isolates abilities to form biofilm were categorized as non-producer, weak, moderate, and strong biofilm producers. The results indicated that the percentages of non-biofilm, weak, moderate, and strong-biofilm activity isolates were 14 (25.46%), 10 (18.2%), 13 (23.64%), and 18 (32.73%), respectively. Out of 55 biofilm and non-biofilm producer *A. baumannii* isolates, 40 (72.73%), 33 (60%), 32 (58.2%), 29 (52.74%) and 14 (25.45%) were positive to the *csuE*, *ompA*, bla_{PER}*-1*, *bap*, and *csgA* genes, respectively (Table 5). In contrast, the *fimH* was not detected in all non-biofilm and biofilm producer isolates. The average for biofilm biomass in *csuE*, *ompA*, bla_{PER}*-1*, *bap* and *csgA* positive *A. baumannii* strains were 0.390 ± 0.184, 0. 329 ± 0.089, 0.431 ± 0.211, 0.329 ± 0.256 and 0.362 ± 0.303, correspondingly.

Statistical analysis exhibited an important association between the incidence of *csuE, ompA*, bla_{PER}*-1, bap* positive strains, and biofilm development in the majority of strains ($P < 0.05$). Our findings exhibited that 72.73% (40 isolates) encoded *csuE* gene and no correlation was detected between this gene and biofilm production ($P \leq 0.001$). In contrast, the existence of *ompA, bap,* bla_{PER}*-1,* and *csgA* genes showed a strong relationship with biofilm produced by 52.73%, 55%, 58.2%, and 25.45% of *A. baumannii* isolates, respectively.

Table 5
Occurrence of biofilm-related virulence genes and biofilm strength in meat *A. baumannii* strains.

Biofilm strength	No of isolates	Biofilm-related genes					
		ompA	***bap***	***bla$_{PER}$-1***	***csuE***	***csgA***	***fimH***
Non-biofilm	14	0	0	0	13	0	0
weak	10	8	9	7	2	7	0
Moderate	13	12	12	15	11	5	0
Strong	18	13	8	10	14	2	0
Total	**55**	**33**	**29**	**32**	**40**	**14**	**0**

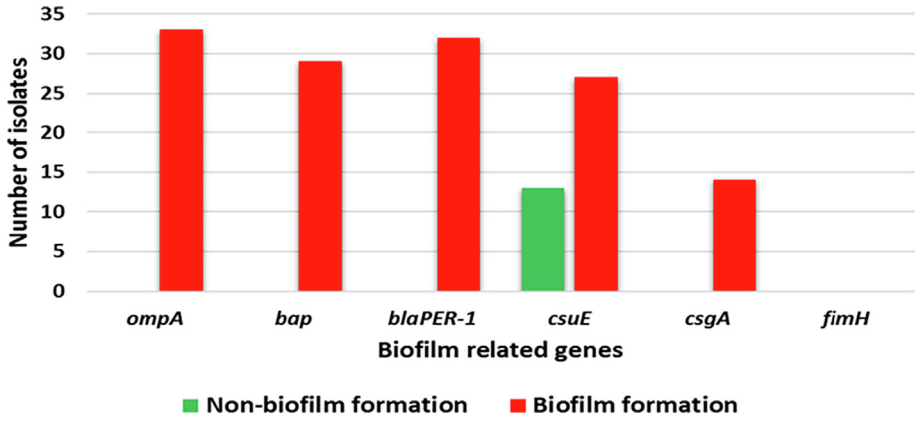

Fig. 3. Frequency of biofilm-related genes in *A. baumannii* strains.

Table 6
Antimicrobial resistance of 55 *A. baumannii* isolates recovered from various meat samples.

Antibiotics used	Raw meat samples (No. of positive samples)								Total Total	
	Camel meat (6)		Cow meat (9)		Sheep meat (27)		Chicken meat (13)			
	No.	%	No.	%	No.	%	No.	%	No.	%
AMP	1	16.67	4	44.44	19	70.70	12	92.3	**36**	**65.45**
PIP	2	33.33	3	33.33	13	48.14	3	23.08	**21**	**38.18**
AMK	0	0	3	33.33	10	37.04	6	46.15	**19**	**34.55**
GEN	3	50	6	66.66	23	85.18	9	69.23	**41**	**74.55**
TOB	2	33.33	6	66.66	15	55.55	6	46.15	**29**	**52.73**
TET	3	50	6	66.66	22	81.48	9	69.23	**40**	**72.73**
AMC	4	66.67	8	88.89	26	96.3	11	84.61	**49**	**89.1**
CAZ	2	33.33	4	44.44	12	44.44	5	38.46	**23**	**41.82**
CEF	1	16.67	3	33.33	3	11.11	4	30.77	**11**	**20**
IMP	2	33.33	2	22.22	6	22.22	2	15.38	**12**	**21.81**

Ampicillin = AMP; Piperacillin = PIP; Amikacin = AMK; Gentamicin = GEN; Tobramycin = TOB; Tetracycline = TET; Amoxicillin/clavulanic acid = AMC; Ceftazidime = CAZ; Cefepime = CEF; Imipenem = IMP.

As shown in Fig. 3, the *ompA*, *bap*, *blaPER-1*, and *csgA* were detected only in biofilm-producing *A. baumannii* isolates. Whereas, the *csuE* gene was identified in both biofilm and non-biofilm forming A. baumannii

3.6. Antimicrobial susceptibility and MAR indices of A. baumannii isolates

Table 6 displayed the degree of antimicrobial resistance of 55 *A. baumannii* strains recovered from various types of uncooked meat samples. Based on our findings, *A. baumannii* isolates of the current investigation revealed a strong resistance against amoxicillin/clavulanic acid (89.1%), gentamicin (74.55%), tetracycline (72.73%), ampicillin (65.45%), tobramycin (52.73%) antimicrobial agents. Whereas; the lowest degree of resistance was noticed against cefepime (20%) and imipenem (21.81%). Statistically substantial variances were observed between kinds of meat specimens and the frequency of antimicrobial resistance in the *A. baumannii* strains ($p < 0.05$). The average MAR index of 55 *A. baumannii* strains in raw meat samples was 0.5. The MAR indies for 2, 4, 16, 9, 17, 6, and 1 isolates of *A. baumannii* were 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8, respectively. The percentage of *A. baumannii* strains with a MAR index > 0.2 was 53/55 (96.36%). Consequently, *A. baumannii* recovered from raw meat is extremely resistant against the majority of antimicrobial agents with extraordinary values of MAR index.

4. Discussion

Recently, Acinetobacter has been developed as a significant pathogen originating in a hospital worldwide (Rebic et al., 2018). Nevertheless, the ubiquitous occurrence of *A. baumannii* in the hospital environment has been represented a routine misunderstanding by numerous studies (Ying et al., 2015; CLSI, 2017; Wang et al., 2017; Rebic et al., 2018), several modern kinds of research have definitely emphasized the survival of this pathogen in numerous types of animal sources (Gurung et al., 2013). In the last decade,

hospitalized infections caused by *A. baumannii*, as an opportunistic microorganism, are increasing (Rebic et al., 2018). Detection and treatment of this pathogen particularly broad-spectrum beta-lactamases and multi-drug resistant strains are of the most important anxiety worldwide.

The current study has mostly been carried out on molecular detection and studying the prevalence rate of *A. baumannii* recovered from various meat samples as well as its ability to produce biofilm and resistance to antimicrobial agents. According to our information, there are scanty reports regarding molecular typing and spreading of virulence factors among the *A. baumannii* strains recovered from the meat of different sources. Fifty-five isolates of *A. baumannii* were recovered out of 220 meat samples collected from slaughterhouses and shopping centers. Based on our findings, sheep meat (46.55%) is the most affected by *A. baumannii* followed by chicken's meat (32.5%) and cow's meat (15%).

In contrast, the fewest isolates were isolated from camel's meat (9.68%). Parallel findings were established by Tavakol et al. (2018) who examined 22 *A. baumannii* strains from 126 raw meat samples and they stated that 45.45% of strains were recovered from chicken meat, 18.18% from bovine meat, 13.64% from camel meat; whereas the lowest frequency of *A. baumannii* was from ovine meat (9.1%). Moreover, former studies revealed that *A. baumannii* has been considered as the animal origin with various disseminations in numerous areas worldwide comprising in Scotland, 1.20% (Hamouda et al., 2011), and Senegal, 5.10% (Kempf et al., 2012). In another investigation performed in Egypt, indicated a high occurrence of *A. baumannii* strains in different foodstuffs of animal origins such as raw meat, raw milk, and dairy products (Rafei et al. (2015). Meat-harboured *A. baumannii* strains isolated from various abattoirs and markets may result in the processing and handling of the meat samples.

Recently, PMFT represents one of the powerful techniques used for identification and discrimination of various types of bacteria and fungi recovered from human and animal samples worldwide (Elbehiry et al., 2019). Accurate identification of *A. baumannii* represents an urgent matter due to its virulence. In the current investigation, PMFT was able to identify 53 out of 55 (96.36%) of *A. baumannii* strains, when we utilized spectra directly from bacterial colonies. Parallel findings were detected by Tavakol et al. (2018), who used MALDI-TOF/MS to identify Acinetobacter species including *A. baumannii,* and found that all tested strains were correctly identified. Another investigation performed by Elbehiry et al. (2017) indicated that MALDI TOF-MS identified all *A. baumannii* (100%) recovered from various food samples. Furthermore, Jeong et al. (2016) exhibited that MALDI Biotyper is a promising, rapid, and precise tool for the detection of Acinetobacter strains at the species and genus levels after modification of the Bruker library.

Several investigations have indicated that the robust existence capability of *A. baumannii* in the surrounding environment and extremely resistant to several antimicrobial agents is mainly caused by the formation of biofilm (Longo et al., 2014; Yang et al., 2019). Not only the relationship between antimicrobial resistance and formation of biofilm in *A. baumannii* were investigated in the current study but also the link between its formation of biofilm and virulence genes *ompA, bap, blaPER-1, csuE, csgA* & *fimH* were included. In our investigation, the interpreted results of Real-Time PCR exhibited that a strong correlation between the frequency of *ompA*, *bap,* and *csgA* positive strains and biofilm formation was detected in the majority of isolates. Our results were matched with Gaddy et al. (2009) who found that *ompA* gene is required for adhesion of *A. baumannii* and somewhat plays a significant role in the production of biofilm (Gaddy et al., 2009).

In addition, our study exhibited that 72.73% of both biofilm and non-biofilm formation *A. baumannii* isolates encoded *csuE* gene and this indicated that there is no correlation between this gene and biofilm formation. Similar findings were interpreted by Lee et al. (2008), indicating that the formation of biofilm in *A. baumannii* was associated with the existence of the *blaPER-1* gene. Likewise, other studies considered that the biofilm formation in *A. baumannii* was connected with the isolates that harboring the *blaPER-1* gene than those that deficient of this gene (Sechi et al., 2004; El-Shazly et al., 2015). Nevertheless, another investigation performed by Bardbari et al. (2017) indicated that *A. baumannii* producing biofilm hasn't connected with the manufacture of *PER-1* β-lactamase. Consequently, a potential clarification for the striking characteristic of *A. baumannii* could be that bla_{PER}-1 rises the attachment of the cells that carry this gene without essentially contributing to the formation of biofilm.

Antimicrobial resistance is considered one of the most significant properties of *A. baumannii*. In the present study, the isolates of *A. baumannii* under the investigation demonstrated a variable degree of resistance against amoxicillin/clavulanic acid (89.1%), gentamicin (74.55%), tetracycline (72.73%), ampicillin (65.45%), and tobramycin (52.73%) antimicrobial agents. Askari et al. (2019) revealed similar findings of antimicrobial resistance against *A. baumannii* recovered from different meat samples. They observed that the resistance of *A. baumannii* against gentamicin, tetracycline, erythromycin, azithromycin, and ciprofloxacin were 87.17%, 79.48%, 74.35%, 66.66%, and 58.97%, respectively. Whereas; additional study achieved by Kiani et al. (2016) illustrated that the incidence rates of antimicrobial resistance against *A. baumannii* recovered from various types of nosocomial infections were 89.55% for tetracycline, 7.46% for tobramycin, 5.97% for amikacin, and 4.47% for imipenem.

A study performed by Ahmad et al. (2018) stated that the frequency rate of antimicrobial resistance of *A. baumannii* recovered from meat specimens were 100% for ampicillin, 20.80% for ceftriaxone, 33.30% for imipenem, 16.60% for gentamicin, 54.10% for kanamycin, 79.10% for tetracycline, 66.60% for chloramphenicol, 100% for trimethoprim. Parallel results were also detected in many parts of the world including Iran (Moradi et al., 2015), Romania (Constantiniu et al., 2004), Turkey (Kulah et al., 2009), France (Kempf & Rolain, 2012) and Italy (Zarrilli et al., 2013).

The MARI is considered one of the hazardous calculation tools. The existing investigation presented MARI fluctuating from 0.2 to 0.8 (average 0.5) which points out that the strains of *A. baumannii* developed from sources of very dangerous contamination. This shows that the excessive usage of antimicrobial drugs led to a higher degree of resistance against *A. baumannii* (Anane et al., 2019). It is believed that the increased prevalence of antimicrobial resistance among different types of bacteria may be due to the intensive use of antimicrobial drugs as a method of therapy as well as for stimulating the growth in food-manufacturing animals, because this increases the antibiotic force for isolates existent (Askari et al., 2019). Otherwise, it is allowed in the Kingdom of Saudi Arabia to use antibiotics only for stimulating the growth. The lower degree of *A. baumannii* resistance against imipenem (carbapenems) and cefepime (4th generation cephalosporins) may be a result of the fact that these antimicrobial agents aren't permitted for the treatment of animals used in the production of food.

Nonetheless, their unlawful prescription produced a significant increase in bacterial resistance against different types of antimicrobial drugs particularly in the chicken's farms. Increasing the rates of antimicrobial resistance in various classes of food-borne pathogens may be due to the high prescription rate of antimicrobial drugs in both humans and animals (Nejat et al., 2015; Ranjbar et al., 2018; Safarpoor-Dehkordi et al., 2018).

5. Conclusions

Our findings revealed that the meat of various animals is considered an important source of *A. baumannii*, especially sheep meat.

PMF represents a robust and rapid technique able to identify approximately 97% of all isolates at the species level. Our findings also revealed that the *ompA*, *bap*, *blaPER-1*, *csgA* are considered biofilm-related genes in *A. baumannii* strains. In contrast, the *csuE* gene wasn't correlated with biofilm formation. The majority of *A. baumannii* strains illustrated strong antimicrobial resistance against amoxicillin/clavulanic acid, gentamicin, tetracycline, ampicillin, and tobramycin. Therefore, the existence of multidrug-resistant *A. baumannii* in meat may represent a clear threat to human health.

Declaration of Competing Interest

There is no conflict of interest or personal relationships that could have appeared to influence the work reported in this paper.

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