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Molecular Detection of Some Virulence-associated Genes in *Acinetobacter baumannii* Isolated from Meat and Dairy Products

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***Abstract*—** This study was conducted to isolate and identify *Acinetobacter baumannii (A. baumannii*) from various types of meat and dairy products; in addition, it studied the distribution of selected virulence-associated genes that are linked to the ability of bacteria to be pathogenic and resistant to antibiotics. A total of 120 samples were taken from meat of sheep, calves, and cow milk products. These samples were cultured onto selective media then confirmed as *A. baumannii* at the species level via the use of the blaOXA-51 gene which is specific for the species. Of the samples tested, 48/120 samples were identified as being positive for *A. baumannii*. In terms of prevalence rates there was no statistical difference observed when comparing samples taken from sheep (42.5%), calf (40.0%), and milk products (37.5%). Virulence associated determinants were also assessed via molecular methods. Of particular note was the detection of *ompA* in approximately 81.2% percent of samples tested. Additionally, the T6SS gene was found to have been present in roughly 47.9% of samples tested. Collectively these data indicate that animals that provide food products (e.g., cows) could represent an important source of potentially virulent strains of *A. baumannii* and further demonstrate how the food supply can function as a vehicle for transmission of this opportunistic pathogen. Additionally, the nearly universal detection of ompA indicates a high prevalence of pathogenic potential amongst the isolated strains. Conversely, the variability in detection of T6SS indicates that virulence mechanisms in *A. baumannii* could be based upon multiple mechanisms dependent upon the individual strain. Collectively, the above findings emphasize the need for continued molecular surveillance of *A. baumannii* within food products produced from animals to ensure adequate food safety and protect public health.

***Keywords* —** *Acinetobacter baumannii, Virulence factors, Outer membrane protein, Type VI transport system (T6SS).*

INTRODUCTION

Food security will continue to be a priority with the world's growing population, in addition to providing for an increasing number of people in terms of quantity, it is also essential that the quality of food produced meets expectations regarding its safety; while animal products such as meat, meat and dairy products, represent a significant portion of the average human's diet, there are many issues affecting both the safety and the sustainability of these products (1,2).

Meat is an important part of many people's diets because it provides valuable nutrition (3). But there are also reports that consumption of meat may have negative impacts on the health and food safety (4). Dairy products from milk supplied people with a great deal of nutritionally rich energy and high-quality proteins as well as numerous other necessary minerals like calcium, magnesium, potassium, zinc and phosphorus, in forms could be quickly absorbed (5). When harmful pollutants get released into the environment, they cause damage to the ecosystem and harm the health of living things, these pollutants can take many forms including chemical, physical, thermal, or microbial pollution. These pollutants contribute to degrading the quality of air, water, and soils (6).

Meat and meat products, milk and dairy products have a variety of bacterial contaminants that can cause serious public health problems (7). These bacteria can enter into meat and meat products through contact with an animals hide or gastrointestinal system (from the gut) or through contaminated equipment and people when they are being processed for slaughter. As a result of this high microbial count, there is potential for rapid spoilage of the product and the risk of foodborne illness will be increased if proper sanitation is not practiced (8). *Acinetobacter* is a Gram-negative, aerobic, non-fermenter, oxidase negative and non-motile bacterium (9). The genus *Acinetobacter* comprises numerous species; however, *A. baumannii* is the most clinically relevant (10).

Acinetobacter species have been identified from either human or animal sources (11). Soil and water contain *Acinetobacter.* Many patients have the organism cultured from their urine, saliva, sputum, tracheal aspirates, and/or open wounds. In addition, the organisms are capable of colonizing

various irrigating solutions and intravenous fluids (12). *Acinetobacter baumannii* has exhibited higher levels of drug-resistant phenotypes compared to its species relatives. Over the last 30 years, strains of *A. baumannii* have demonstrated increasing resistance to newer antimicrobial agents. These antimicrobial agents are used for treatment of infections in both humans and animals (13).

Acinetobacter produces a variety of proteins that causes antibiotic resistance through efflux systems, and/or degradation (14). The overproduction of efflux pumps decreases the concentration of an antibiotic reaching its target. Six clinically relevant families of efflux pump have been found in the *Acinetobacter* spp. (15).

The Type VI Secretion System (T6SS), especially in pathogenic *A. baumannii*, works as a molecular syringe for interbacterial competition and virulence. Through T6SS, it provides bacteria with the ability to deliver toxins from one cell to another cell including *E. coli*; this provides the advantage for bacterial survival in polymicrobial environments as seen in infection (16). This study aimed to investigate the incidence *A. baumannii* in meat, meat products, milk and dairy products, and study the presence of some virulence factors that associated with antibiotics resistance.

MATERIALS AND MTHODS

A) Isolation and identification of bacteria:

1) Out of 120 totally samples, collected from 40 samples from each of sheep and calf (10 from each of meat, minced meat, liver and kidney), as well as 40 samples from cows' milk products (divided into 10 samples were taken from raw milk, cheese, yogurt and cream). The samples were taken by swabs and kept in transport media, and transported to lab as soon as possible,

2) These samples were prepared and cultured on different media such as (MacConkey agar, blood agar, nutrient agar), incubated for 18 hours in 37º C. For identification *A. baumannii.* As well as using specific primer for molecular identification.

B) Specific primers used for molecular detection on efflux pump and type VI transport system as a predictive virulence factors related to these bacteria.

Using specific primer for species-specific gene *bla-OXA-51* with F: AATGCTTGATCGGCCTTG, R: TGGATTGCACTTCATCTTGG (353 bp) (17) and the virulence gene for outer membrane protein with the sequence F: CGCTTCTGCTGGTGCTGAAT, R: CGTGCAGTAGCGTTAGGGTA (530 bp) (18), and type 6 transport system F: ATCGGCGTTTGTTCTTACG, R: CGTTACTGCCGATCGAATC (420 bp) (19). Following a 5-minute denaturation at 94°C, two sets of amplification cycles were conducted: the first set consisted of 20 cycles and the second of 10 cycles. For the first set (loop 1), each cycle involved denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, extension at 72°C for 2 minutes, and a final incubation at 72°C for 10 minutes (20).

C) Statistical Analysis:

Across this study SPSS program used to examine Chi-square test of independence (χ^2), Fisher's exact test, and Pearson correlation coefficient (r) had used for statistical tests and p-value <0.05 was considered statistically significant.

Ethical Approval:

The research was carried out according to ethical principles. The protocol of this study, along with the subject information and consent form, underwent scrutiny, and received approval from a local committee ethics; identified by the reference number (UOK. VET. HE.2025.166).

RESULT AND DISCUSSION

Prevalence Rate of *Acinetobacter baumannii*:

Out of 120 totally samples, collected from 40 samples from each of sheep and calf (10 from each of meat, minced meat, liver and kidney), as well as 40 samples from cows' milk products (divided into four groups (10 samples each) of raw milk, cheese, yogurt, and cream.). The results presented in Table (1) reveal a similar prevalence of *A. baumannii* among the three groups (sheep, calves and milk products) and there is no statistically significant difference among the groups. Thus, it may be assumed that these three groups serve as comparable reservoirs within the food chain.

Table 1. Prevalence rates of *Acinetobacter baumannii*, in the studied groups with overlapping 95% confidence intervals

Groups	Positive	Total	Positive %	95% CI	P value
Sheep	17	40	42.5	28.5-57.8%	0.9011
Calves	16	40	40.0	26.3-55.4%	
Milk Products	15	40	37.5	24.2-53.0%	
Total Positive 48 isolates; Negative 72 (Other types of bacteria or no growth)					

P value ≤ 0.05 is significant

These findings support previous studies reporting that *A. baumannii* has been detected on a variety of foods (raw meats and milk), creating an external (non-human) source for its potential spread into communities (21). The lack of statistical differences between *A. baumannii* isolates obtained from sheep, calves, and milk products in our study suggests that interventions should target the entire production chain rather than a single animal species (22). *A. baumannii* was isolated from sheep, cow and camel meats, while there were higher levels of *A. baumannii* in sheep, the presence of the bacterium on each of the other types of meats was strongly suggests of cross-contamination among all animal species (23).

Studies of raw milk have shown that *A. baumannii* has been isolated at approximately 17-28%. This indicates that milk is an important medium for the dissemination of *A. baumannii* and a possible route of zoonosis (24). In addition, the fact that there are no statistically significant differences among the sample groups indicates either a similarity in the processing/handling practices applied to each of your sample groups, or that there is a common contamination pathway

(e.g., equipment, environment, personnel) that affects all groups similarly (25).

Compared to the above prevalence rates (approximately 38-43%), the prevalence rate in current study is relatively high; however, it is consistently high regardless of the group. Therefore, it appears that this study supports the concept that *A. baumannii* is widespread throughout the animal-food interface, as suggested by research that demonstrates raw meat and milk may serve as community reservoirs and potential vehicles for multi-drug-resistant strains of *A. baumannii* (26).

Molecular Detection of *Acinetobacter baumannii*:

Out of 120 samples taken, 48 isolates had the intrinsic *blaOXA-51* gene based on molecular detection. The results obtained was confirm that strains belong *A. baumannii* because *blaOXA-51* has been recognized as a species-specific intrinsic gene marker for *A. baumannii*. The results shown in Table (2) and Figure (1).

Table 2. Molecular detection of *A. baumannii* based on *blaOXA-51*

Gene	Positive (n)	Negative (n)	Positive %	Negative %
bla-OXA-51	48	72	40 %	60 %

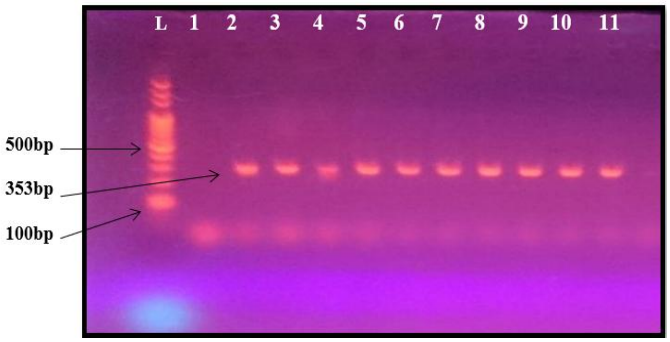

Figure 1. Gel electrophoresis of 2% agarose for PCR *blaOXA-51* products visualized under UV light after staining with ethidium bromide.; lane 1: negative, lane 2-11: positive with 353 bp. L: 100 bp marker

The findings of this study supported by previous studies where blaOXA-51 was identified genotypically and confirmed *A. baumannii* isolates, supporting its reliability for molecular identification of *A. baumannii* from both human clinical samples and non-human environmental samples (27).

Turton *et al.*, (28) demonstrated blaOXA-51-like was found in all of *A. baumannii* isolates but none were detected in the other non-baumannii *Acinetobacter* species; therefore, proposed it for use as a species-specific molecular identifier for *A. baumannii*. Since then, routine clinical and environmental studies have used blaOXA-51-like PCR to identify A. baumannii isolates with 100% carriage reported among confirmed isolates (27).

Detection of Acinetobacter baumannii Associated virulence Factors:

Outer Membrane Protein A (ompA) Gene:

Beginning with the *ompA* gene, it was found to be present in 81.2% (39/48) of the isolates examined. Conversely, *ompA* negative results were obtained for 18.8% (9/48) of the isolates. The results shown in Table (3) and Figure (2).

Table 3. Molecular detection of ompA gene in *A. baumannii*

Gene	Positive (n)	Negative (n)	Total tested	Positive %	Negative %
omp A	39	9		81.2%	18.8%

As *ompA* is a key outer membrane porin involved in biofilm formation, cell adhesion and invasion into host cells, as well as providing the bacteria with an ability to resist serum and deliver cytotoxic substances through outer membrane vesicles, these data suggest that nearly all of the food derived *A. baumannii* isolates in this study have some level of virulence potential (29).

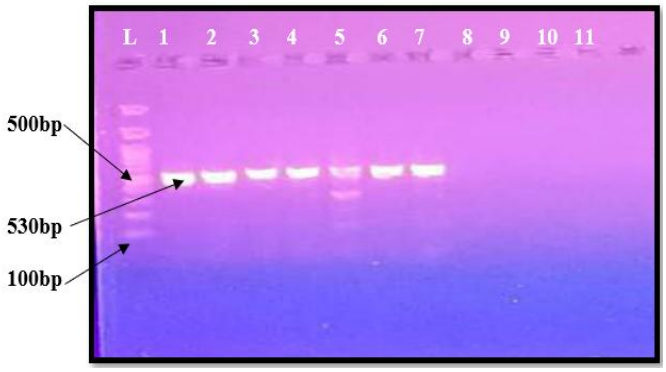

Figure 2. Gel electrophoresis of 2% agarose for PCR *ompA* products visualized under UV light after staining with ethidium bromide.; lane 1-7: positive, lane 8-11: negative with 530 bp. L: 100 bp marker

Previous research has demonstrated the presence of *ompA* is strongly correlated with increased levels of biofilm formation, multiple drug-resistant phenotypes as well as increased virulence in clinical isolates of *A. baumannii* (30). The fact that 81.2% of the meat and milk isolates contain *ompA*, suggests that the non-clinical (extrahuman) strains found in the food chain may also possess the same virulence factors which contribute to their ability to cause infection in humans and reinforce the necessity of monitoring and controlling *A. baumannii* in animal derived products through a one health approach (31).

Taking into account these factors, the *ompA* positive isolates from the environment/food may represent a high level of environmental/food strains that have virulence potential similar to those of clinical isolates at least through OmpA mediated mechanisms (32). And given their ability to form biofilms on surface and equipment and likely cause infection when transmitted to a host they are consistent with the concept that meat and dairy products can be reservoirs of virulent *A.*

baumannii (33). The ompA acts to mediate adherence and invasion of epithelial cells as well as protect bacteria from both serum and complement mediated killing. OmpA is released through outer membrane vesicles where it can induce host cell apoptosis and cytotoxicity resulting in damage to the tissue (34). Reviews that are current have emphasized the importance of OmpA as a primary virulence factor and that has been identified as a potential vaccine and/or drug target for its high concentration and conserved function within pathogens (35).

Type 6 Secretion System (T6SS):

Nearly half of the tested isolates (47.9%, n=23) had a type VI secretion system-associated determinant, T66S, while approximately half (52.1%, n=25) did not; as shown in Table (4) and Figure (3). In addition, this represents a widespread but non-universal occurrence of T6SS machinery in *A. baumannii*, as evidenced by the fact that nearly half of the isolates contain T6SS-related machinery.

Table (4): Molecular detection of *T6SS* gene of secretion system in *A. baumannii*

Gene	Positive (n)	Negative (n)	Total tested	Positive %	Negative %
T6SS	23	25	48	47.9%	52.1%

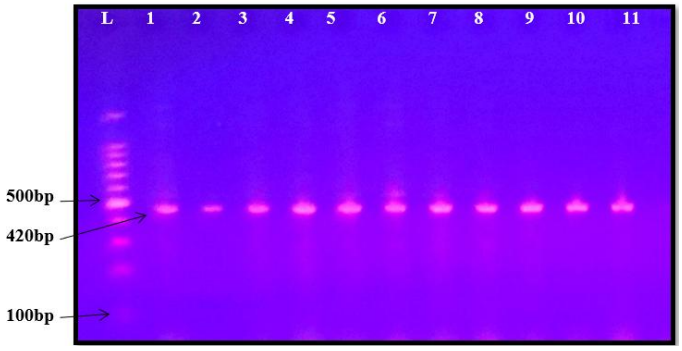

Figure 3. Gel electrophoresis of 2% agarose for PCR *T6SS* gene products visualized under UV light after staining with ethidium bromide.; lane 1-11: positive with 420 bp. L: 100 bp marker

A well-documented mechanism by which type VI secretion systems contribute to the ecological success and competitive capacity of bacteria is through the mediation of interbacterial competition. Additionally, type VI secretion systems have been implicated in virulence associated characteristics in *A. baumannii*, such as serum survival, and in the case of certain strains, antimicrobial resistance adaptation (36). Therefore, the presence of T66S in 47.9% of the tested isolates, in conjunction with the lack of this determinant in the majority (approximately 53.1%) of the tested isolates provides further evidence that the type VI secretion system is both strain specific and not a necessary component of persistence in all *A. baumannii* lineages (37).

Many isolates of *A. baumannii* could benefit from having a T6SS system in order to have an advantage over competing bacteria in environments where there are many types of bacteria present (food, tissue of animals, etc.) however some isolates may be able to thrive without one (38). This is evidenced by improved the ability of these to interact with competing bacteria, survive in serum, and adapt to conditions in order to cause infection (39).

Oh *et al.,* (29) also, noted that the isolates that did not have the T66S gene cluster; this suggests that even though these isolates do not have T6SS, they can still be successful as *A. baumannii* due to alternative methods of interaction with their environment like biofilm formation, OmpA mediated adhesion, efflux pumps and antibiotic resistance genes.

Dong *et al.,* (37). examined the presence of the T6SS gene cluster in 77 clinical isolates of *A. baumannii* and reported that the T6SS was identified in 51 of those isolates and that there was a significantly greater prevalence of T6SS in clinically resistant isolates compared to susceptible isolates. The authors linked the presence of T6SS to both resistance to antibiotics and to increased competition among bacteria. Moreover, Li et al., (39) demonstrated that, the key genes involved in the function of T6SS were more prevalent in drug resistant isolates and that the two specific genes tssM and tssD contributed to virulence, survival in serum and to various phenotypes related to resistance (40).

CONCLUSION

In conclusion, this study clearly demonstrates that *A. baumannii* can be isolated from both meat and dairy products. From a total of 120 samples tested, we obtained forty-eight confirmed isolates. There was no statistical difference in isolation rate among sheep, calves, and milk products. Molecular characterization of our isolates utilized *blaOXA-51* and virulence profiling of these isolates indicated a high frequency of *ompA* and a moderate occurrence of *T66S*.

Thus, our findings suggest that food-borne *A. baumannii* may express certain characteristics that would allow them to be persistent in a host environment; enable colonization; and possibly result in a risk to public health due to consumption of contaminated food. Although all of the *ompA* positive isolates had a high degree of pathogenic potential genes, the sporadic nature of *T6SS* detection implies that virulence in *A. baumannii* may depend upon additional strain-specific mechanisms. Therefore, in order to prevent contamination and ultimately inhibit the spread of potentially virulent *A. baumannii* in food products derived from animals; enhanced sanitary practices during food production and preparation should be implemented in conjunction with continuing routine microbiological/molecular monitoring of these food products.

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