Original Paper

Predictive assessment of *Arcobacter butzleri* in retail beef and mutton meat.

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ABSTRACT

Arcobacter species are Gram-negative rods that have been implicated in food and water borne diseases. The purpose of this study is to clarify *Arcobacter butzleri* that has been identified as a pathogen causing foodborne disease. Arcobacter is considered as a food borne emergent pathogen able to cause disease among human and animals. Concern is growing over the widespread presence and high incidence of Arcobacter in food, since consuming polluted food and water is thought to be the main important way of Arcobacter spreading to human being. In this study, a total of 100 random fresh retail beef and mutton (50 of each) samples were purchased from different butcher's shops in Benha city, Kalubia Government, Egypt. *A. butzleri* was recorded at 3/50 and 4/50 of examined beef and mutton samples. The seven isolated strains of suspected *A. butzleri* were biochemically identified and then subjected to PCR confirmation and revealed that presence of some putative virulence genes of *A.butzleri* such as fibronectin-binding protein gene (*cad*F) and Iron-regulated outer membrane protein gene (*irg*A). The strains were also examined for antibiotic sensitivity and showed that strains were highly resistance to Streptomycin and were sensitive to Meropenem.

1. INTRODUCTION

Fresh meat is considered as a particularly perishable food due to its high content in protein, along with a variety of endogenous antioxidants, vitamins, minerals, and other bioactive molecules like carnitine, taurine, carnosine, ubiquinone, and creatine (Shaltout, 2001; Devatkl et al., 2004 and Hughes et al., 2015).

Food sources, especially those of animal origin have been majorly implicated in *Arcobacter* infection in humans, implying successful colonization at primary production, contamination during slaughter operations and survival in food products once contaminated has taken place (Shange, 2020).

Arcobacter butzleri strains have been discovered in a variety of matrices, including dairy, meat, and vegetables, demonstrating their adaptability to environmental varieties. The metabolic traits gained over by evolving of (7,474genes) pangenomic variety, emphasized by Isidro et al. (2020).

Also, genus Arcobacter, formerly known as "aerotolerant *Campylobacter*" was included into the family *Campylobacteraceae*. They are a spore free, slow-growing, mobile, spiral, and Gram-negative bacterium (Vandamme and De Ley, 1991).

Thirty- three identified species of Arcobacter have been isolated from various sources, *A. butzleri, A. cryaerophilusin* addition to *A. skirrowii* were reported to cause a possible risk tohuman (Fera et al., 2008 and Shah et al., 2011). The most frequently discovered species of Arcobacter, particularly in foods, is *A. butzleri,* followed by *A. cryaerophilus* and *A. skirrowii.* (Collado et al., 2009).

Arcobacter spp. causes bacteremia, endocarditis, peritonitis, gastroenteritis, and diarrhea in human being as well as mastitis, diarrhea, and abortion in animals. (Ramees et al., 2017).

Even though the number of *Arcobacter* spp.-related illnesses has increased recently, little is known about their pathophysiology (Collado and Figueras, 2011). Using the housekeeping 13 genes utilized by Pérez-Catalua et al. (2018), ANI, AAI, DDH and RAxML genome-based analyses. (Fanelli et al., 2020)20 *A. butzleri* genomes were subjected to taxogenomic analysis, using 16S rRNA gene sequence analysis. This theory has to be validated by more *A. butzleri* genomes, which should be extracted from various environmental niches, sequencing, and analysis are done as different subspecies of *A. butzleri* are used to describe it and show where the isolation came from.

The aim of the present work is to isolate *Arcobacter butzleri* from different fresh beef and mutton samples, clarify their incidence, antimicrobial resistance to make a further identification using PCR technique and finally to conclude their public health importance in human being.

2. MATERIAL AND METHODS

2.1. Samples:

One hundred randomly selected samples of retail beef and mutton, represented by 100g of each sample, were purchased from different butcher's shops (50 of each) from Benha city, Kalubia Governorate, Egypt. The samples were stored in an ice box and kept apart in sterilized plastic bags, then labeled and transferred under complete aseptic conditions to the laboratory as rapidly as possible. The collected samples were examined to detect their

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contamination with Arcobacter species particularly *Arcobacter butzleri.*

2.2. Isolation of Arcobacter species (ISO 4833-1, 2013):

Twenty-five gm of examined sample were homogenized with 225 ml of 0.1% sterile peptone water from which 1 ml was transferred into 9 ml of Arcobacter-specific broth (ASB) represented with H- broth (Oxoid, Basingstoke, United Kingdom). The homogenate was mixed well and inoculated with 5-fluorouracil (100 mg), trimethoprim (64 mg), novobiocin (32 mg), cefoperazone (16 mg) and amphotericin B (10 mg) and incubated aerobically in the broth, for 24 hours (Houf et al., 2001).

After incubation, H-medium was streaked with 20 ul of enrichment broth, this had the same five antibiotics as H-broth but was in a solid form. supplement (Houf broth and Houf plates "HH method"). At 30°C, the inoculating plates underwent an aerobic incubation for 48 hours. Up to 10 colonies were then selected based on their morphology (Gram-negative, round, small gray-white colonies) and streaked onto Columbia agar containing 5% (vol/vol) defibrinated horse blood and incubated for 48 hours. The developed colonies were picked up, purified, and inserted for further identification onto slope Nutrient agar tubes.

The presumed isolates of Arcobacter species were identified in accordance with MacFaddin (2000), then subjected for further identification either morphologically according to ISO (1995) or biochemically according to OIE (2008) were performed as seen in (Table A).

Table A phenotypic characteristics of Arcobacter spp.

Characteristics	Positive	Negative
Motility	**	
Catalase activity	**	
Oxidase	**	
Hippurate hydrolysis	**	
Thermostable nuclease	**	
Hydrogen sulphide production	**	
Sugar fermentation	Appearance of pink color	
Methyl red		**
Voges-praskauer (VP)		**
Urease		**
Growth in 2% Nacl	**	

2.3. Antibiotic sensitivity of Arcobacter species:

Employed a single diffusion approach to determine antimicrobial resistance in accordance with Elmali and Can (2017) for Arcobacter species. The susceptibility of the isolated Arcobacter strains were determined by different concentrations of Sensitivity discs *(*Oxoid Limited, Basingstoke, Hampshire, UK*)*. The bacterial culture was evenly distributed throughout the nutrient agar surface, then over the surface of the infected plate, the antibiotic discs were positioned. Moreover, the plates were incubated at 25°C for 2-7 days and then checked their growth enclosing the antibiotic discs. The zone of maximum inhibitory activity for the bacterial growth revealed the maximum effect of the antibiotic on the growth of the bacteria. Therefore, The National Committee for Clinical Laboratory Standards was followed when conducting the antimicrobial susceptibility test. "NCCLS" (2001) as shown in (Table B).

2.4. Polymerase Chain Reaction (PCR):

Arcobacter strains were subjected to molecular analysis to confirm their identification and genetic profiles. Polymerase chain reaction was conducted to confirm the isolates belonging to *Arcobacter butzleri*, specific 16S rRNA fragments for *A. butzleri* were used to characterize and demonstrate such strains (Houf et al., 2000) (Table C). Also, to identify the presence of fibronectin-binding protein gene (*cad*F) and iron-regulated outer membrane protein gene (*irg*A) as virulence factors of *A. butzleri* was performed as seen in (Table,4). DNA extracted utilizing QIA amp kit (Shah et al., 2009), then make amplification of Arcobacter species as well as amplification of *cad*F *and irg*A genes of *A. butzleri* (Lehmann et al., 2015). The amplified products were detected by electrophoresis in 3% agarose gels and visualized with UV transillumination (Sambrook et al., 1989).

Table B Antimicrobial discs, concentration, and interpretation of their action on the isolated Arcobacter species.

Antimicrobial agent	Sensitivity disc content (ug)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Amikacin (AK)	30	12 or less	13-15	16 or more
Meropenem (M)	10	9 or less	10-12	13 or more
Neomycin (N)	30	12 or less	13-16	17 or more
Erythromycin (E)	15	13 or less	14-22	23 or more
Gentamicin (G)	10	12 or less	13-14	15 or more
Ipipenem (IPM)	10	18 or less	19-21	22 or more
Ciprofloxacin (CP)	5	15 or less	15-19	20 or more
Ampicillin (AM)	10	13 or less	14-17	18 or more
Cefepime (FEP)	30	18 or less	19-24	24 or more
Streptomycin (S)	10	11 or less	12-14	15 or more
Levofloxacin (L)	5	18 or less	19-21	22 or more
Clindamycin (CL)	10	13 or less	14-16	17 or more
Cefotaxime (CF)	30	17 or less	18-22	23 or more
Tetracycline (T)	30	14 or less	15-18	19 or more
Nalidixic acid (NA)	30	13 or less	14-18	19 or more
Sulphamethoxazol (SXT)	25	10 or less	11-15	16 or more

Table C Sequences of Primer of some Arcobacter for PCR system.

Fragment	Primers	Oligonucleotide sequence (5′ → 3′)	Product size(bp)	References
A. butzleri 16S rRNA	BUTZ (F)	5′CCTGGACTTGACATAGTAAGAATGA′3		
	ARCO (R)	5′ CGTATTCAACCGTAGCATAGC ′3	401	Houf et al. (2000)
	*cad*F(F)	5′ TTACTCCTACACCGTAGT ′3	283	
	*cad*F (R)	5′ AAACTATGCTAACGCTGGTT ′3		Douidah et al. (2012)
	*irg*A(F)	5′TGCAGAGGATACTTGGAGCGTAACT ′3	437	
	*irg*A (R)	5′ GTATAACCCCATTGATGAGGAGCA ′3		

2.5. Statistical analysis:
Statistically evaluation of the obtained results was performed using the Analysis of Variance (ANOVA) test (Feldman et al., 2003).
Control positive of *A. butzleri* was obtained from Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, Osaka, Japan. In contrast, *E. coli* strain was used as a negative control.

3. RESULTS

Classification and recognition of isolated Arcobacter species, revealed that the prevalence of *Arcobacter butzleri* in beef in addition to mutton specimens was 7% from the totally examined 100 samples and it was 6% in beef while 8% in mutton samples as shown in (Table 1).
Arcobacter species were subjected to antimicrobial susceptibility by the single diffusion method, and they were evaluated as susceptible, intermediate, and resistant.
Arcobacter spp. were highly resistant to streptomycin and erythromycin, intermediate to tetracycline while it was highly sensitive to gentamicin, cefepime and meropenem as shown in (Table 2).
Antimicrobial resistance profile of *A. butzleri* against (16) antibiotics cleared that highly resistance of *A. butzleri* strains against streptomycin and erythromycin, intermediate against nalidixic acid and cefotaxime, while it was sensitive to cefepime and meropenemas shown in (Table 3).
Furthermore, the presence of some putative virulence genes of *A. butzleri* strains isolated from examined samples such as fibronectin-binding protein gene (*cad*F) and Gene for the

outer membrane protein controlled by iron (*irg*A) were recorded, so, *cad*F gene was detected in 7 (100%) of the examined strains of *A. butzleri*, while *irg*A gene was detected in 5 (71.4%) of the isolated strains as shown in (Table 4).

Table 1 Frequency of *Arcobacter butzleri* isolated from the examined retail beef and mutton samples (n=50).

Meat Arcobacter Spp.	Beef No.	%	Mutton No.	%	Total (100) No.	%
Arcobacter butzleri	3	6	4	8	7	7

Table 2 Resistance to antibiotics of Arcobacter species found in examined retail beef and mutton samples (n=18).

antimicrobial substance	S NO	%	I NO	%	R NO	%
Streptomycin (S)	-	-	-	-	18	100
Erythromycin (E)	-	-	1	5.7	17	94.4
Clindamycin (CL)	2	11.1	3	16.7	13	72.2
Sulphamethoxazol (SXT)	4	22.2	2	11.1	12	66.7
Nalidixic acid (NA)	3	16.7	5	27.8	10	55.6
Cefotaxime (CF)	6	33.3	2	11.1	10	55.6
Ampicillin (AM)	8	44.4	1	5.7	9	50.0
Ciprofloxacin (CP)	8	44.4	2	11.1	8	44.4
Tetracycline (T)	7	38.9	4	22.2	7	38.9
Neomycin (N)	9	50.0	2	11.1	7	38.9
Amikacin (AK)	11	61.1	-	-	7	38.9
Levofloxacin (L)	12	66.7	1	5.7	5	27.8
Ipipenem (IPM)	12	66.7	2	11.1	4	22.2
Gentamicin (G)	14	77.8	-	-	4	22.2
Cefepime (FEP)	15	83.3	1	5.7	2	11.1
Meropenem (M)	17	94.4	-	-	1	5.7

Table 3 Antimicrobial resistance profile of A. butzleri isolated from the examined retail beef and mutton samples (n=7).

NO	Strains	Antimicrobial resistance profile	MAR index
1	*A. butzleri*	S, E, CL, SXT, NA, CF, AM, CP, T, N, AK, L, IPM, G, FEP, M	1
2	*A. butzleri*	S, E, CL, SXT, NA, CF, AM, CP, T, N, AK, L, IPM	0.812
3	*A. butzleri*	S, E, CL, SXT, NA, CF, AM, CP, T, N, AK, L, IPM	0.812
4	*A. butzleri*	S, E, CL, SXT, NA, CF, AM, CP, T, N, AK	0.687
5	*A. butzleri*	S, E, CL, SXT, NA, CF, AM	0.438
6	*A. butzleri*	S, E, CL, SXT	0.250
7	*A. butzleri*	S, E	0.125
Average	0.589		

Table 4 Occurrence of virulence genes of A. butzleri isolated from the examined retail beef and mutton samples (n=50)
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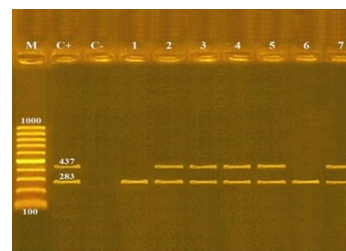| Virulence genes		*cad*F	*Irg*A
Meat	No. of isolates	No	No
Beef	3	3	2
Mutton	4	4	3
Total	7	7	5

Figure 1 Multiplex PCR of the virulence genes cadF (283 bp) and irgA (437 bp) on Agarose gel electrophoresis for A. butzleri. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive of A. butzleri for cadF and irgA genes. Lane C-: Control negative. Lanes 1 and 6: Positive A. butzleri strains for cadF gene. Lanes 2, 3, 4, 5 and 7: Positive A. butzleri strains for cadF and irgA genes.

4. DISCUSSION

Arcobacter incidence in food is rising and the majority of species exhibit antibiotic resistance, so, this study attempts to look into the incidence of *A.butzleri* and their antimicrobial resistance in examined fresh meat samples collected from butcher's shops, Benha city, Kalubia government , Egypt.

The data obtained from this study was lower than that obtained by Dufy and Fegan (2012) who examined 100 beef samples 20 (20.0%) of the export abattoirs were positive for Arcobacter spp. as recorded in (Table 1).

In addition, higher isolation rate of Arcobacter spp. from fresh beef samples (55.6 and 22%) were obtained by Vytrasova et al. (2003) and Rivas et al. (2004), respectively, while lower isolation rate (2.2%) was obtained by Kabeya et al. (2004).

As reported by Collado and Figueras (2011) and Unver et al. (2013) utilizing disc diffusion tests and Cervenka et al. (2006) who observed that all the tested strains were intermediately susceptible to Erythromycin, the data from (Table 2) are consistent with low resistance against Erythromycin.

On the contrary, the results recorded by Elmali and Can (2017) revealed that the most effective antibiotic was Tetracycline, because 96.66% of the isolates were susceptible against it.

Furthermore, data illustrated in (Table 4) agree with Parisi et al. (2019) who looked into the virulence of 10 *A.butzleri* strains and they found that 100% of the strains carried *cad*F, *cia*B, *tly*A, *mvi*N, *pld*A and *cj*1349 virulence factors genes; 10% *hec*B; 50% *irg*A; 60% *iro*E.

Additionally, earlier work done by Zacharow et al. (2015) on the genetic diversity of *A. butzleri* in beef and chicken , revealed that 100% of the strains of *A. butzleri* presented *tly*A gene, 90% contained *cad*F, 71% had *cj*1349, 100% encoded*cia*B, 53% contained *irg*A, 34% had *hec*A, 48% encoded *hec*B and 93% contained *pld*A.

A. butzleri has been linked to gastrointestinal illness on numerous instances both in demographic research and clinical investigations (Vandenberg et al., 2004; Abdelbaqi et al., 2007, Kownhar et al., 2007; Kopilovic et al., 2008 and Jiang et al., 2010). The main *A.butzleri* symptoms was persistent watery diarrhea.

Recurrent stomach cramps without diarrhea was the major symptom of an *A.butzleri* epidemic that affected 10 kids in an Italian school. The infection was severe enough to need the hospitalization of 3 kids (Vandamme et al., 1992). The fourth most frequent Campylobacter-like bacteria discovered in patients with diarrheal feces was *A.butzleri.* There has been speculation that an epidemic persistent stomach pain in a school in Italy was caused by person-to-person (PTP) transmission of the bacteria *A. butzleri.* (Vandamme et al., 1992).

5. CONCLUSION

In conclusion, *A. butzleri* is vastly distributed throughout the food and it recorded as the highest incidence among Arcobacter species. Meropenem and Cefepime were recorded as the highest antimicrobial resistance against the *Arcobacter butzleri* strains and so considered the drug of choice.

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