

Inhibitory Effect of Cinnamon and Thyme Essential Oils Against *Acinetobacter baumannii* strains Isolated from Raw Milk and Some Milk Products

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Abstract

A total of 250 random samples including raw milk samples collected from street vendors, dairy shops and dairy houses in addition to some milk products including Kariesh cheese, Domiati cheese, cream, and milk powder, were collected from different areas in Luxor city, Egypt. for detection of *Acinetobacter species* by conventional method and PCR for the presence of *bla_{ox51} like gene*. results revealed that the 10% of *Acinetobacter spp.* were recovered from whole raw milk samples, species were *A. baumannii*, *A. calcoaceticus* and *A. haemolyticus* with percentages of 5.3, 2.7 and 2%, respectively. street vendors raw milk samples showed the highest prevalence (14%) of *Acinetobacter spp.* Regarding milk products samples, 8% of *Acinetobacter spp.* were isolated from kariesh cheese. A higher percentage of 25% were recorded from cream samples. All *Acinetobacter baumannii* strains harbored *bla_{ox-51} like gene*, showing strong lipolytic activity on tributyrin agar, the antibacterial effect of cinnamon oil and thyme oil against 16 *Acinetobacter baumannii isolates* showed high antibacterial activity.

Keywords: *Acinetobacter species.*, Dairy products, PCR, *bla_{ox51} like gene*, Essential oils

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INTRODUCTION

Raw milk may be contaminated with various bacteria that have influence on its quality and safety (Kable et al., 2016). *Acinetobacter spp.* are the predominate deteriorating bacteria, which can produce heat-stable enzymes as proteases and lipases, that, remain active even after pasteurization, and can spoil the milk during long storage. (Fusco et al., 2020). *Acinetobacter spp.* are belonging to family *Moraxellaceae*, strictly aerobic, Gram-negative, non-fermenting bacteria, omnipresent in nature and not only occurs in the soil, water, foods, but also dairy products (Balows et al., 1992; Gurung et al., 2013). Some *Acinetobacter spp.* were detected in refrigerated raw milk and milk products (Munsch-Alatossava and Alatossava, 2006; Hantsis-Zacharov and Halpern, 2007; Rafei et al., 2015; Saad et al., 2018).

Several *Acinetobacter spp.*, especially *A. baumannii*, possess multiple-drug resistance (MDR) phenotype (Amorim et al., 2017). Recently It has been appeared as a clinically relevant pathogen which cause massive range of nosocomial infections in hospitals world-wide leading to high mortality (Peleg et al., 2008) and (Antunes et al., 2014). Also, *non baumannii Acinetobacter* as *A. calcoaceticus* that is recorded as an environmental species, has been demonstrated in many cases of bacteremia and pneumonia (Mostachio et al., 2012), also nosocomial infections by *A. lwoffii*, *A. junii*, or *A. johnsonii* were also recorded (Lee et al., 2007; Karah et al., 2011).

The extend of antibiotic-resistant bacteria in food constitutes a crucial public health interest as many human pathogens may be transferred through food chain (Lupo, et al., 2014). The of *Acinetobacter*

spp. in raw milk and its products was reported by Von Neubeck et al., (2015) and Cho et al., (2018).

The *bla_{OXA-51-like}* genes were present in all *A. baumannii* species so that, their detection supply simple and convenient method of identification on the species level (Turton et al., 2006). Some microorganisms in milk and dairy products produce lipases that cause bitter taste, rancidity and gelling, Marchand et al., (2009) and Von Neubeck et al., (2015). Lipolytic enzymes produced by *Acinetobacter* and other bacteria present in dairy foods may affect the taste, flavor, texture and odor of the products (Koohi et al., 2014 and (Pangallo et al., 2014).

Recently antibacterial activity of essential oils is of great interest due to their effect on multidrug resistant bacteria (Mo and Os 2017; Soliman et al., 2017 and Vasireddy et al., 2018). Divers researches have estimated the inhibitory effect of plant essential oils against *A. baumannii* (Salman et al., 2008; Costa et al., 2009; Rosato et al., 2010; Jazani et al., 2011; Duarte et al., 2012 and Sonbol et al., 2017).

Therefore, this study was applied to illustrate the incidence of different *Acinetobacter spp.* in raw milk and some dairy products through isolation, identification and detection of their lipolytic activity. As well as to assess the antibacterial effect of two essential oils (cinnamon and thyme oil) on the selected strains of *A. baumannii*.

MATERIALS AND METHODS

I. Sampling

A total of 250 samples of milk and some dairy products were dividing as 150 raw milk samples from street vendors, dairy shops and dairy farm houses (50 samples each) and 100 samples of Domiati cheese, kariesh cheese, cream and milk powder (25

samples each) were gathered from various locations within Luxor city, Egypt, the samples were transported to the laboratory expeditiously. The samples were prepared according to APHA, (1992).

II. Isolation and identification of *Acinetobacter* species.

a- Enrichment

One ml or 1g of the samples was aseptically inoculated into sterile 10 ml nutrient broth cotton plugged test tube, and the broth was incubated at 37°C / 24-48 h (Bollet et al., 1995).

b- Selective plating and isolates identification

Aliquots of the previously incubated broth cultures were streaked onto plates of Leeds *Acinetobacter* agar medium and incubated at 37°C / 24-48 h. (M1839-HiMedia TM, India) supplemented with multidrug resistant *Acinetobacter* selective supplement (FD 271-HiMedia TM, India, and then incubated at 37°C / 24-48 h. Pink colonies with mauve background were recorded as *Acinetobacter* spp. (Jawad et al., 1994). Then Gram negative bacilli biochemically examined: catalase test (MacFaddin, 2000), oxidase test (Baron et al., 1994), lysine decarboxylase, indole Test, Triple Sugar Iron Agar medium (TSI), nitrate reduction (Kreig and Holt, 1984), gelatin hydrolysis (Clarke and Cowan 1952), citrate utilization (Koneman et al., 1992), phenylalanine deaminase (Baron et al., 1994), haemolysin production (Cowan, 1974) and growth at different temperature (Kwan and Skura, 1985). interpretation as recommended by (Bouvet and Grimont 1987).

III. Detection of *A. baumannii* by PCR for the detection of *bla*_{OXA-51}-like gene.

a) DNA extraction & PCR amplification

DNA extraction from samples was completed using the QIAamp DNA Mini kit (Qiagen, Germany) according to the manufacture's recommendations. The DNA amplification was performed using the oligonucleotide primers as mentioned by Woodford et al., (2006).

Primer	The Target gene	Oligonucleotide sequence (5' → 3')	Product size
OXA-51-like (F)	<i>bla</i> _{OXA-51-like} gene	5' TAATGCTTTGATCGGCCTTG 3'	353 (bp)
OXA-51-like (R)		5' TGGATTGCACTTCATCTTGG 3'	

b) PCR reaction and thermal cycling parameters

The reaction was conducted in thermal cycler (Biometra) (Master cycler, Eppendorf, Hamburg, Germany) in Animal Health Research Institute (AHRI), Dokki, Giza, Egypt. The cycling parameters were: Denaturation at 94°C for 3 min, followed by 35 cycles at 94°C /45 secs, annealing at 57°C /45 secs, elongation at 72°C /1 min and extension at 72°C /5 min. The PCR reaction was carried out in a mixture of 25 µl. The reaction mix consisted of 6 µL of Template DNA, 4.5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl₂, 2 µl of 10mM dNTP mix, 1 µl each of forward and reverse primer (10 pmol) and 2.5U of Taq DNA polymerase. PCR products were screened by electrophoresis on 1.5% agarose gel stained with ethidium bromide and visualized and captured under ultraviolet light (Sambrook et al., 1989).

IV. Detection of lipolytic activity of the isolated *Acinetobacter baumannii*, (Harrigan and McCance, 1976).

Prepared tributyrin agar plates were streaked by overnight cultures and incubated at 30°C / 3 days. The medium appeared opaque, but a clear zone surrounded the lipolytic colonies.

V. Estimation of antibacterial activity of (cinnamon and thyme essential oils) against selected *A. baumannii* strains by Agar Disk Diffusion Method.

Antibacterial activity of cinnamon and thyme essential oils was estimated using the disk diffusion assay described by Bauer et al., (1966). Therefore, the isolates were overnight cultured (37°C / 24 h) in Mueller Hinton Agar (MHA) and the bacterial suspensions were performed with Mueller Hinton Broth to match McFarland standard No. 0.5 (1.5×10^8 cfu/mL). 100 µl of this suspension were spread on MHA plates. Sterile paper discs (6 mm diameter) were soaked with 15 microliter of the selected essential oils and placed on the surface of MHA plates. These plates were incubated at 37°C / 24 h. Duplicates of the experiments were done, and the average of these values were the results (Kaskatep et al., 2016). The diameters of the inhibition zone were measured in mm and the organism was classified as resistant, intermediate and sensitive. Negative control was done using distilled water. Interpretation of results of antibacterial clear (inhibitory) zone diameters (NCCLS 2012; Oliveira et al., 2013).

Results	Average diameter of clear(inhibitory) zones (mm)	Symbol
Resistant(R)	≤ 8 mm	(-)
Sensitive(S)	9-14 mm	(+)
Very Sensitive	15-19 mm	(++)
Extremely Sensitive	≥ 20 mm	(+++)

RESULTS

From results recorded in Tables (1 & 2) it was obvious that 30 samples of milk and dairy products were contaminated with *Acinetobacter*. fifteen strains could be isolated from the examined raw milk samples (10%) and 15 strains from the examined dairy product samples. In this study numerous *Acinetobacter spp.* were recovered as *A. baumannii*, *A. calcoaceticus*, *A. haemolyticus*, and *A. iwoffii*

The results obtained in Table 1 revealed that for raw milk samples, the highest incidence of *Acinetobacter* was detected in Street vendors raw milk (14%), while 10% and 6% were obtained from dairy shops and dairy farm samples respectively.

Table 1. Incidence of different *Acinetobacter spp.* in the examined raw milk samples

Types of raw milk samples	Positive samples		<i>Acinetobacter spp.</i>					
			<i>A. baumannii</i>		<i>A. calcoaceticus</i>		<i>A. haemolyticus</i>	
	No./50	%	No./50	%	No./50	%	No./50	%
Street vendors	7	14	3	6	2	4	2	4
Dairy shops	5	10	3	6	2	4	-	-
Dairy farm	3	6	2	4	-	-	1	2
Total	15	10	8	5.3	4	2.6	3	2

From raw milk samples (8) strains (5.3%) were identified as *A. baumannii*, divided as 6, 6 and 4% from Street vendors, dairy shops and dairy farm raw milk samples respectively. four strains (2.6%) were found to be as *A. calcoaceticus* from Street vendors, dairy shops raw milk samples (4% for each). Moreover, 3 strains (2%) were identified as *A. haemolyticus* from Street vendors and dairy farmhouses raw milk samples 4% and 2%, respectively.

The results obtained in Table 2 showed that from dairy products samples, the highest incidence of *Acinetobacter* was obtained from fresh cream samples (24%). In addition to 8, 16 and 12% were isolated from kariesh cheese, domiati cheese, and milk powder samples, respectively.

Table 2. Incidence of different *Acinetobacter* spp. in the examined milk products samples

Types of samples	Positive samples		<i>Acinetobacter</i> spp.									
			<i>A. baumannii</i>		<i>A. calcoaceticus</i>		<i>A. haemolyticus</i>		<i>A. iwoffii</i>			
	No./25	%	No./25	%	No./25	%	No./25	%	No./25	%	No./25	%
Kariesh cheese	2	8	1	4	-	-	-	-	1	4	-	-
Domiati cheese	4	16	1	4	3	12	-	-	-	-	-	-
Cream	6	24	4	16	-	-	2	8	-	-	-	-
Milk powder	3	12	2	8	1	4	-	-	-	-	-	-
Total	15	15	8	8	4	4	2	2	1	1	-	-

From dairy products samples *A. baumannii* was isolated from 4, 4, 16 and 8% of kariesh cheese, domiati cheese, fresh cream and milk powder samples, respectively, *A. calcoaceticus* was detected in 12 and 4 from domiati cheese and milk powder samples, *A. haemolyticus* were isolated of 8% from fresh cream samples. *A. iwoffii* were recovered from 4% of kariesh cheese samples.

From data reported in Fig. a illustrated that 16 strains were harbored *bla_{OXA51}* like gene by conventional PCR, which confirmed that these strains were *A. baumannii*.

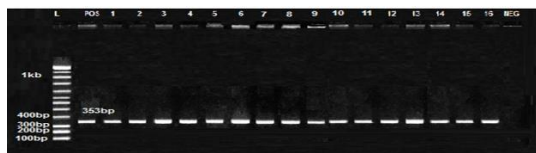


Figure a: Detection of *A.baumannii* by PCR for identification of *bla_{OXA-51}*-like gene. Lane 1-16. positive result of *bla_{OXA51}* gene detection in *A. baumannii* isolates. - (1.8) raw milk, (9) Kariesh cheese, (10) Domiati cheese, (11.12) Milk powder, (13.16) cream. Lane Pos. positive control strain.

Lane Neg. negative control. Lane L.100bp DNA ladder as molecular size DNA marker.

From the result recorded in Table 3 and Fig. b, it was obvious that all *Acinetobacter* strains showed strong lipolytic activity. *A. baumannii*, *A. calcoaceticus*, *A. haemolyticus*, and *A. iwoffii* showed lipolytic activity with percentages of 53.3, 26.66, 16.66% and 3.33%, respectively.

Table (3). Lipolytic activity of different *Acinetobacter* spp. isolated from raw milk and some milk products

Source of strains	Tested strains	<i>Acinetobacter</i> spp.							
		<i>A. baumannii</i>		<i>A. calcoaceticus</i>		<i>A. haemolyticus</i>		<i>A. iwoffii</i>	
		No.	%	No.	%	No.	%	No.	%
Raw milk	15	8	53.3	4	26.66	3	20	-	-
Kariesh cheese	2	1	50	-	-	-	-	1	50
Domiati cheese	4	1	25	3	75	-	-	-	-
Cream	6	4	66.6	-	-	2	33.33	-	-
Milk powder	3	2	66.6	1	33.33	-	-	-	-
Total	30	16	53.3	8	26.66	5	16.66	1	3.33



Figure b: lipolytic activity of *A. baumannii* strains on tributyrin agar show zone surround the colonies

From the result reported in the Table 4 and Fig.c, it was clear that cinnamon and thyme essential oils had a powerful antibacterial effect on the tested strains of *A. baumannii*.

DISCUSSION

Acinetobacter spp. is considered a cause of nosocomial infections worldwide (Almasaudi, 2018). Many *Acinetobacter spp.* were previously isolated from raw milk and milk products (Saad et al., 2018) and (Hoque et al., 2019).

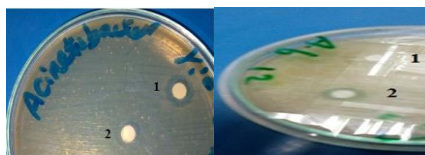


Figure c :Antibacterial effect of essential oils on *A. baumannii* strains show zone of inhibition. 1-Zone of inhibition surround thyme oil disc. 2-Zone of inhibition surround cinnamon oil disc.

In this study, Table 1 showed the incidence of *Acinetobacter spp.* in the examined raw milk, it is clear that 15 isolates were detected as *Acinetobacter spp.* and were classified as, 7 samples (14%) of street vendors's, 5 samples (10%) of dairy shop's, 3 samples (6%) dairy farm.

Table (4). Diameter of inhibition zone (mm) by cinnamon and Thyme oil against *A. baumannii* strains.

Diameter of inhibition zone (mm)	<i>A. baumannii</i> strains No.	Cinnamon oil Thyme oil	
		Cinnamon oil	Thyme oil
Resistant	≤8 mm	Not Detected	Not Detected
Sensitive	9.14 mm	Not Detected	Not Detected
Very sensitive	15.19 mm	2	Not Detected
Extremely sensitive	≥20mm	14	16
Total		16	16

Some investigations showed lower incidence of *Acinetobacter spp.* in raw milk (Uraz and Çitak, 1998; Jayarao and Wang, 1999; Gurung et al., 2013) and (Al Atrouni et al., 2016), they found that *Acinetobacter spp.* were detected in 1.1%, 4.5%, 1.3%, 7.7% and 5.2% of the examined samples, respectively, and one isolate could be identified by Nörnberg et al., (2010).

On the other side, other investigations showed higher results of *Acinetobacter spp.* as identified by Amer et al., (2005), Awad et al., (2005), Amer et al., (2008), Shin et al., (2013), Von Neubeck et al., (2015), Alrazakkazal and Abdullah (2016), Saad et al., (2018) and (Hoque et al, 2019). They found that *Acinetobacter spp.* were detected in 95, 16, 24, 19.6, 23, 62, 16, 15 and 52.9% from the examined raw milk samples, respectively.

As well, 10% of the examined raw milk samples were polluted with *Acinetobacter spp.*, which included *A. baumannii.*, *A. calcoaceticus* and *A. haemolyticus* with percentages 5.3, 2.7and 2%, respectively, indicating higher incidence of *A. baumannii*. *A. baumannii* was detected in 6% of the examined street vendor's raw milk samples, along with *A. calcoaceticus* and *A. haemolyticus* (4% each). Concerning dairy shop's raw milk samples *A. baumannii* and *A. calcoaceticus* were detected in 6% and 4%, respectively. *A. baumannii* and *A. haemolyticus* were detected in 4% and 2% respectively from dairy farms raw milk samples.

The high percentage obtained from street vendor's raw milk samples may be due to the natural habitats of *Acinetobacter* in water, soil and environment and may be due to contaminated tanks Fournier et al., (2006). Detection of *Acinetobacter spp.* from dairy shop's raw milk samples can be due to the most common sources of *Acinetobacter* in milk as inefficient cleaning of dairy equipment, storage and transport of milk, and biofilm as concluded by Santana et al., (2004). The detection of *Acinetobacter spp.* from dairy farms due to contaminated milking places, teat, and dust which are sources for *Acinetobacter spp.* contamination of milk (Vacheyrou et al., 2011). Wani et al., (2006) mentioned that

Acinetobacter spp. is recognized as a normal flora of animals and human skin besides being present in soil, water and sewage.

Regarding the examined dairy products samples, it was found that, 15% *Acinetobacter spp.* were recovered from 100 milk products samples and were categorized as follow 8%. were isolated from Kareish cheese, 16% from Domiati cheese, 24% from fresh cream and 12% milk powder samples.

On the species level, Table 2 showed that Kareish cheese samples were free from *A. calcoaceticus*. while, *A. haemolyticus* was absent in the examined Kareish, Domiati cheese and milk powder samples. *A. iwoffii* could not be detected in Domietta cheese, fresh cream and milk powder samples. Kareish cheese is a traditional cheese produced in Egypt from raw milk. The contamination of the Kareish cheese with *Acinetobacter spp.* may be due to the bad handling and cutting during processing in farmers houses and the contamination from soil, the primitive way of production and selling and the use of raw milk (Saad et al., 2018).

Many studies reported *Acinetobacter spp.* in cheese samples (Awad et al., 2005; Amer et al., 2008; and Xue et al., 2018). Contamination of Domiati cheese with *Acinetobacter spp.* is a result of using of raw milk or inefficient heat-treated milk for the production of the cheese or post pasteurization contamination during the production and the handling of the cheese. Moreover, the unhygienic method of selling of Domiati cheese could be a major reason of contamination in the refrigerator of the groceries where various types of foods are put closely to each other. (Saad et al., 2018).

Higher results of cheese contamination were detected by (Awad et al., 2005 and Saad et al., 2018) they mentioned that 20 and 13.3% of *Acinetobacter spp.*, could be isolated from Kareish cheese samples respectively. Also, they found that 24% of *Acinetobacter spp.*, could be isolated from samples of Domiati cheese, but lower result of 3.3% *Acinetobacter spp.*, was present in Domiati cheese samples obtained by (Saad et al., 2018)

Regarding the examined cream samples, the obvious contamination can be due to use of raw contaminated milk, improper handling during manufacture, bad storage or unhygienic containers in the refrigerator as *Acinetobacter spp.* are psychrotrophic. Recently, Saad et al., (2018) mentioned that for the examined cream samples, *Acinetobacter spp.* was isolated (13.3%), and *A. baumannii* (10%) and *A. haemolyticus* (3.3%) were identified.

In this study 12% of milk powder samples were contaminated with *Acinetobacter spp.* including *A. baumannii* (8%) and *A. calcoaceticus* (4%). Higher results 42 isolates obtained by Cho et al., (2018) and the predominant isolated species were *A. baumannii*, *A. pittii*, and *A. calcoaceticus*.

It is obvious from the results that the most isolated species were *A. baumannii*, in both examined raw milk (5.3%) and milk product samples (8%). lower incidences of 3.6, 2.7 and 6.97% were obtained by (Shin et al., (2013), Rafei et al., (2015) and (Vithanage et al., 2016), respectively. Therefore, Al Atrouni et al., (2016), could not isolate *A. baumannii* from the examined samples.

On contrast, Higher results of 14.2%, 32.4%, 27. 8% and 10% were postulated by

Hantsis-Zacharov and Halpern (2007); Gurung et al., (2013); Kim et al., (2017) and Saad et al., (2018), respectively.

Concerning milk products samples *A. baumannii* was isolated *equally* from kariesh and Domiati cheese samples (4% for each), while a high percentage of 16% was detected in fresh cream samples and 8% was reported in milk powder samples.

Higher result (14.3%) was obtained by (Rafei et al., 2015) who could isolate one (14.3%) of *A. baumannii* from seven cheese samples. Lower results were recorded by Li et al., (2017), they isolated that *A. baumannii* (1.09%) across six cheese samples collected from two different artisanal factories of Kazakhstan. Lower results were detected also by Saad et al., (2018), who identified *A. baumannii* 3.3% in Domiati cheese samples, however higher results were reported from Kareish cheese 10%.

The obtained results indicated that 8% of the examined milk powder samples were contaminated with *A. baumannii*, higher results were obtained by Cho et al., (2018). *A. baumannii* in cream samples represents 16 %, was lower result of 10% obtained by Saad et al., (2018).

PCR assay for the detection of *blaOXA-51-Like* gene was performed for the detection of *blaOXA-51-like* gene, which confined only on *A. baumannii* and was amplified at a 353-base-pair (bp) amplicon, as an internal gene for molecular confirmation of *A. baumannii* isolates at the species level. In this study all *A. baumannii* strains (16) that diagnosed conventionally and biochemically were also positive for that *blaOXA-51-like* gene photo (1), this prove that gene is a tool for differentiation of *A. baumannii*. Therefore, we confirmed that *blaOXA-51-like* gene is considered as

a reliable marker for identification of *A. baumannii*.

All *Acinetobacter* isolates from the examined raw milk and some dairy products, had lipolytic activity. These results agreed with that of Franciosi et al., (2011) as almost whole *Acinetobacter spp.* displayed powerful lipolytic activity by tributyrin agar plates Saad et al., (2018) concluded that 85.2% of *Acinetobacter* strains from the examined milk and dairy products, showed lipolytic activity. Lower incidences from raw milk 3.7% and dairy products 66.7% were demonstrated by Amer et al., (2005), respectively. Another study by Amer et al., (2008) concluded that 81.8 % of *Acinetobacter spp.* were isolated from raw cow milk and 50% were recovered from white soft cheese which had lipolytic activity.

A. baumannii has been considered as a red alert microbe due to its ability to resist extensive used antibiotic (Howard et al., 2012). So, the direction of researches became towards searching for other agent with antibacterial effect on this resistant pathogen.

The current study also evaluated the antibacterial effect of cinnamon and thyme oil against examined strains of *A. baumannii*, isolated from raw milk and milk products in this study, the highest inhibitory zone diameter was 32 mm. by thyme oil and 30 mm by cinnamon oil. all the sixteen *A. baumannii* strains tested showed zone diameters of 19 mm or above (very sensitive), this show the significant effectiveness of thyme and cinnamon oil as antibacterial. Also, thyme oil is more effective against *A. baumannii* isolates from dairy origin (Sonbol, et al., 2017). These results agreed with many studies on the antibacterial effect of thyme oil against *A.*

baumannii as mentioned by Hammer et al. (1999) they found that Thyme oil was among the most effective out of 52 essential oils against *A. baumannii*. Trojanowska, et al., (2016) indicated that *in vitro* antibacterial activity of thyme oil was determined by the disc diffusion method and the inhibition zones of various oil concentrations against *A. baumannii*, ranged from 7 to 44 mm. different types of essential oil were tested against *Acinetobacter spp.*, thyme oil had a higher effect on all tested *Acinetobacter* isolates (Abdel Salam et al., 2018).

(Łysakowska, et al., 2011) confirmed that thyme oil showed powerful activity towards both standard and clinical strains of *Acinetobacter spp.* at low concentrations.

Concerning cinnamon oil, the obtained result was in consistence with (Kaskatep et al., 2016) they evaluated the effect of cinnamon essential oil against *A. baumannii*. They determined higher zone diameters (44 mm). (Al-janabi and Yaseen (2018) found that Cinnamon essential oil was the most effective against *Acinetobacter spp.*, and at 1.2 dilution they obtain highest zone of inhibition at 44mm while, minimum inhibition zone was measured at 30 mm at 1.8 dilutions.

Many studies recommended the applicable use of different essential oils as cinnamon and thyme oil in the dairy industry as food dietary supplement with improving quality parameters Georgescu et al., (2018) and Saad and Abdel-Salam (2015). As well, Hachana et al., (2019) claimed that thyme essential oil can be used as a simple alternative solution for production of farm cheese without needing heat treatment, while ensuring high nutritional value food product.

CONCLUSION

The aforementioned data proved that raw milk and dairy products are major sources for transition of *Acinetobacter spp.* especially *A. baumannii* strains to food chain with harmful effects on human health. Also these bacteria responsible for production of heat-stable lipases, which become active also after pasteurization and thus can spoil the milk and milk products, the obtained data also confirm the antibacterial effects of thyme and cinnamon oil against multidrug resistant *A. baumannii* strains, hence this study suggest production of raw milk and milk products mixed with these essential oils. Further studies needed to evaluate the antibacterial effect of other available essential oils against other multidrug resistant bacteria.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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