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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF FOODBORNE BACTERIAL PATHOGENS ISOLATED FROM *BAGHLAVA* AN IRANIAN EXPORTING PASTRY SWEET

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ABSTRACT

Baghlava, a traditional pastry sweet product, are manufactured in Iran and exported to different countries around the world known as a tasty confectionery. The aim of this study was to investigate the prevalence and antibiotic resistance pattern of foodborne pathogens isolated from *Baghlava* samples. *E. coli*, *C. sakazakii*, *Salmonella* spp., *C. perfringens* and *S. aureus* were isolated and identified using PCR assay for detection of virulence factor gene in *Baghlava* samples. All pathogens except *Salmonella* spp. were detected in samples. Total contamination rates of *E. coli*, *C. sakazakii*, *C. perfringens* and *S. aureus* were observed 8.92, 7.14, 1.78 and 2.67%, respectively. Multidrug resistance properties to amoxicillin and ampicillin have been found in all strains; however, all isolates were susceptible to ciprofloxacin. Hierarchical clustering and contamination patterns of pathogens showed that the prevalence of each pathogen is significantly higher in the southern and northern regions of the city than central areas in which these products were produced.

1.Introduction

Baghlava is a traditional sweet product categorized as a hard confectionery mostly are just produced with an especial formulation in Qazvin and Kermanshah cities in Iran but consumed around the world as a tasty sweet confectionary (Mahmoodi Sadr et al., 2019). Also, this sweet is produced with a different formulation in some other areas of the world such as Turkey and Arabian countries (Krondl, 2011). The principle ingredients for *Baghlava* producing in Qazvin with specific formulation are nuts powder including pistachio and almond, egg, milk, sugar, crushed saffron and cardamom powders. After crushing all components, the initial pastes of the *Baghlava* is formed then punched layer by layer (consisting of pistachio, almond and cardamom pastes separately) into the specific pan containers following cooking process at 180 °C for 30 min in the oven. Finally, it is cut into the small pieces then covered with concentrated sugar syrup for being fresh during the storage time (Gharibzahedi, 2018). This traditional product is consumed in Iran and also exported to many countries in Asia and Europe (Mahmoodi Sadr et al., 2019). Using different ingredients in *Baghlava* manufacturing make it sensitive to spoilage and increase the risk of foodborne pathogen transmission to consumers

as a vehicle. On the other hand, cooking process and higher osmotic pressure as a key characteristic of this product reduce the probability of bacterial viability, growth and activity (Lee et al., 2011).

Foodborne bacterial pathogens are the strains of bacteria transmitted to humans by food consumption leading to intestinal and extraintestinal diseases and disorders. Consumption of contaminated food with foodborne pathogens is associated with enteric infections and several outbreaks annually around the world (Paudyal et al., 2017). There are many foodborne pathogens identified in traditional and ethnic pasty sweets and confectionaries. Staphylococcus aureus, Escherichia coli serotype O157: H7 and *Salmonella* spp. have the most prevalence reported by researchers in traditional products (Grace, 2015). Other foodborne pathogens such as Clostridium perfringens and Cronobacter sakazakii also have recently been detected in pastry ethnic confectionaries (Matheus et al., 2016). Some of these pathogens can survive during thermal processing and low water activity condition of sweet products; Consequently, the transmission risk of these pathogens, unfortunately, threaten the health of consumers (Mahmoodi Sadr et al., 2019). Presence of virulence factor encoded genes is crucial for risk assessment and evaluation the prevalence of pathogens isolated from food samples. Ordinarily, identification and confirmation of foodborne pathogen presence in food samples are implemented by detection of the most important virulence factor gene known for each pathogen using polymerase chain reaction (PCR) procedure (Law et al., 2015). It is worth pointing out that the region of traditional food production and sample collection significantly affect the prevalence of isolated pathogens as previously described by many researchers. The contamination rates reported significantly different for traditional food samples collected from manufacturers and local markets located in southern, northern and central regions of the city (Owusu-Kwarteng et al., 2017).

Multidrug resistance characteristics of pathogens isolated from food samples attract the attention of researchers in recent decades. Several studies investigated and detected phenotypic properties of antimicrobial resistance in pathogens isolated from food samples representing serious concern for the public health (Deak et al., 2016). Due to the extensive use of antibiotics in medical cares and agricultural process, development of resistant foodborne pathogens has been increased rendering less effective treatment for infections in human (Baym et al., 2016). Investigation of antimicrobial susceptibility pattern of isolated pathogens can help to develop the strategies for figuring out ways to solve these problems (Porse et al., 2016). This research has been done to investigate the prevalence and antimicrobial susceptibility pattern of some foodborne pathogens isolated from *Baghlava* sweet samples collected from manufacturers and local markets located in southern, northern and central regions of Qazvin city, Iran.

2. Materials and methods

Totally 112 *Baghlava* samples were randomly collected from local markets located in different areas of Qazvin, Iran; from May to September 2018. Samples were immediately transported under cool condition to the laboratory of Health Products Safety Research Center, Qazvin University of Medical Science and stored at 4 °C until primary microbial isolation procedures. All *Baghlava* samples showed normal organoleptic and physical properties consisting of colour, odour and consolidation.

2.1.Antibiotic susceptibility testing

Antibiotic resistance pattern of isolates was investigated using Kirby-Bauer antibiotic testing method as described by the Clinical Laboratory and Standards Institute (CLSI, 2016). As described in the procedure, confirmed isolates colonies were transferred and grown on Muller-Hinton agar (Promedia, Spain) for disk diffusion technique. Antibiotic susceptibility of isolates was studied against ampicillin (AM)(10

µg/disk), amoxicillin (AMX)(30 µg/disk), chloramphenicol (C)(30 µg/disk), tetracycline (TE)(30 µg/disk), ciprofloxacin (CP)(5 µg/disk) and ceftriaxone (CRO)(30 µg/disk). The diameters of inhibition areas (mm) were measured for evaluation antibiotic susceptibility profile of the strains. The results were reported as resistant (R), intermediate sensitive (I) and complete sensitive (S) according to the CLSI interpretation guidelines. Reference organisms for quality control of the antimicrobial susceptibility testing procedure were employed, including Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922.

2.2. Culture-base microbial isolation and identification

At the present study, *Escherichia coli, Cronobacter sakazakii, Salmonella spp., Clostridium perfringens, and Staphylococcus aureus* were initially isolated and identified through collected samples using culture-based isolation and identification methods to detect presumptive isolates describing in Microbiological Examination methods of Food and water. For sample preparation, twenty-five grams of each sample was homogenised and blended for 1 min using Stomacher BagMixer Lab-blender (Interscience, France) then mixed with 225 mL of sterile buffered peptone water (BPW, ProMedia, Spain); subsequently, they were subjected to initial isolation based on culture methods (Da Silva et al., 2018).

2.3. DNA extraction

For extraction of genomic DNA, presumptive confirmed single colonies of bacterial isolates were picked up from each isolation medium for inoculation into 5 mL of Bovine Heart Infusion broth (BHI-broth, ProMedia, Spain) for 24 h at 37 °C using 120 rpm shaking. Incubated medium tubes were centrifuged at 5000g for 15 min. After supernatant separation, the biomass pellets were subjected to DNA extraction by commercial kit. Cinnagen DNA extraction kit for gram-negative bacteria (Cinnagen Co. Iran) was used in accordance with the manufacturer instructions. NanoDrop spectrophotometer (ThermoFisher Scientific Co., USA) was employed for quantity and quality evaluation of extracted strain genomes. DNA samples were stored at -20 °C until the PCR assay.

2.4. PCR assay

PCR assay was employed to confirm the presence of *E. coli, C. sakazakii, Salmonella, C. perfringens* and *S. aureus* between the strains isolated and presumptively identified from *Baghlava* samples by culture base method. Species-specific primers for detection of rfb (Hu et al., 1999), *ompA* (Kilonzo-Nthenge et al., 2012), sty (Kim et al., 2006), *plc* (Abildgaard et al., 2010) and *nuc* (Kim et al., 2001) genes, present in *E. coli, C. sakazakii,* Salmonella, *C. perfringens* and *S. aureus* respectively, were used at the present study. Primer sequences and thermal cycling procedures for each primer are provided in Tables 1 and 2. PCR mixtures consisting of 200 µM of each dNTP, 10 mM $(NH_4)_2$ SO_4, 2.5 U taq polymerase, 3 µM $MgSO_4$, 10 mM Tris-HCl (Cinnagen Co. Iran), 70 ng DNA template, 3 µM of each primer and addition of deionized sterile water reaching the final volume of 25 µL were used for each PCR reaction. PCR products were characterised using electrophoresis at 100 V for 1 h on a 1.5% agarose gel containing 0.005% v/v safe staining dye (Ampliqon, Denmark).

Table 1. Primer sequences for PCR assay

Primers	Accession Number	Sequence	Amplicon size (bp)	Reference
rfbEF	S83460.1	GTGTCCATTTATACGGACATC CATG	292	Hu et al. 1999
rfbER		CCTATAACGTCATGCCAATATTGCC		
ompAF	NZCP011047.1	GGATTTAACCGTGAACTTTTCC	369	Kilonzo-Nthenge et al. 2012
ompAR		CGCCAGCGATGTTAGAAGA		
sty1F	U25352.1	TGGTATGGTTAAGCGGAGAATGG	424	Kim et al. 2006

sty1R		GAGAGTCATAGCCCACACCAAAG		
plcF	NC008261.1	GCTAATGTTACTGCCGTTGA	325	Abilgaard et al. 2010
plcR		CCTCTGATACATCGTGTAAG		
nucF	AF400161.1	CGAAAGGGCAATACGCAAAG	310	Kim et al. 2001
nucR		CGTAAGCCACGTCCATATT		

Table 2. Thermal cycling procedures of PCR assays

Foodborne pathogen	Primer	Thermal cycling program
E. coli O157: H7	rfbE	Initial denaturation: 5 min at 94°C, followed by 35 cycles: 40 s at 94°C, 60 s at 59°C and 60 s at 72°C; finally, 4 min at 72°C as final extension step
C. sakazakii	ompA	Initial denaturation: 5 min at 95°C, followed by 30 cycles: 60 s at 95°C, 60 s at 55°C and 45 s at 72°C; finally, 5 min at 72°C as final extension step
Salmonella spp.	sty	Initial denaturation: 5 min at 94°C, followed by 40 cycles: 40 s at 95°C, 30 s at 61°C and 35 s at 72°C; finally, 7 min at 72°C as final extension step
C. perfringens	plc	Initial denaturation: 3 min at 94°C, followed by 40 cycles: 60 s at 94°C, 60 s at 57°C and 50 s at 72°C; finally, 3 min at 72°C as final extension step
S. aureus	nuc	Initial denaturation: 5 min at 95°C, followed by 45 cycles: 60 s at 95°C, 60 s at 58°C and 60 s at 72°C; finally, 6 min at 72°C as final extension step

2.5. Statistical analysis

Fisher`s exact and Chi-square tests were carried out for evaluating the significant differences ($P < 0.05$) between contamination rates using SPSS software version 22.0.1 (Chicago, IL, USA). Heatmap and hierarchical clustering were performed by RStudio version 1.2.1335 and R package version 2.8.1 available on www.rstudio.com and www.r-project.org websites respectively. All statistical and experimental measurements were performed in triplicate.

3.Results and discussions

3.1. Prevalence of foodborne pathogens

Identification of pathogenic *E. coli*, *C. sakazakii*, *Salmonella* spp., *C. perfringens* and *S. aureus* strains were implemented by culture-based methods in 11, 10, 0, 6 and 5 samples respectively confirmed by the morphology of typical colonies and biochemical tests. As they are provided in the Figures 1-4, presence of rbfE, *ompA*, *plc* and *nuc* genes was confirmed by PCR assay in 10, 8, 2 and 5 *E. coli*, *C. sakazakii*, *C. perfringens* and *S. aureus* isolates respectively. Consequently, total contamination rate (including all samples collected from different areas of the city) of confirmed *E. coli*, *C. sakazakii*, *Salmonella* spp., *C. perfringens* and *S. aureus* strains isolated from *Baghlava* samples were determined 8.92 (2.67% from southern, 3.57% from northern and 2.67% from central areas of the city), 7.14 (3.57% from southern, 2.67% from northern and 0.89% from central areas of the city), 0, 1.78 (0.89% from southern and same contamination rate from central areas) and 2.67% (0.89% from northern and 1.78% from central areas) respectively (Fig. 5). Total contamination rate by *E. coli* and *C. sakazakii* were significantly higher than that for other foodborne pathogens in collected *Baghlava* samples from different areas of the city. As it is illustrated in Fig 5, *C. sakazakii* and *E. coli* for samples collected from southern and northern areas; *S. aureus* and *E. coli* for central areas had significantly higher contamination rate than other pathogens in samples collected from each areas of the city. As it can be seen in the Fig. 6, the contamination rate pattern of the southern and northern areas of the city was

significantly categorised in one group (cluster 2). Indeed; because of higher hygienic inspection of the manufacturers located in the central areas of the cities, lower microbial contamination rate generally observed in samples collected from this area as described by other researchers. Yang et al. in the year 2016 found that the prevalence of foodborne pathogens in food samples collected from northern areas is higher than other regions. Also, the prevalence patterns of *E. coli* and *C. sakazakii* as gram negative foodborne pathogens (considering absence of *Salmonella* spp.) clustered in the same group (cluster A) showing significant correlation between prevalence of these pathogens in *Baghlava* samples.

Baghlava is a sweet product with lower water activity and moisture content led to declination in growth rate and pathogenicity risk of a broad spectrum of foodborne pathogens; however, presence and transmission of some pathogens including *Salmonella,* Clostridium, Staphylococcus, Escherichia coli and Cronobacter species are still probable and previously reported by some researchers in the same sweet products. At the present study, prevalence and antimicrobial susceptibility pattern of these probable foodborne pathogens were evaluated. All foodborne pathogens except *Salmonella* spp. were detected in 112 *Baghlava* samples.

Prevalence of *Salmonella* spp. is more reported in poultry and seafood products (Issa et al., 2017); however, detection and survival of this pathogen have been dispatched by many researchers in sweet and confectionary products recently (Nascimento et al., 2018). Negative results for detection of *Salmonella* spp. in *Baghlava* samples reveal this product is safe regarding the absence of the most dangerous foodborne pathogen. Also, according to the national food safety standards of Iran, *Salmonella* spp. must be absent from twenty-five grams of any food and drink samples (Mahmoodi Sadr et al., 2019). *Salmonella* spp. usually transmit to the confectionary and sweet products by some ingredient and additives consisting of nuts and its powder (Woh et al., 2017).

Regarding use of almond and pistachio nut powder as raw materials in *Baghlava* formulation, contamination of these components with *Salmonella* spp. can be transmitted to the final product effectively. Farakos et al. (2017) and Harris et al. (2016) reported considerable prevalence and levels of *Salmonella* spp. contamination in almond and pistachio nut samples respectively (Farakos et al., 2017, Harris et al., 2016). Cooking process during *Baghlava* manufacturing should be a probable reason for not detecting of pathogens (Manios & Skandamis, 2015).

E. coli and *C. sakazakii* as gram negative foodborne pathogens were detected in *Baghlava* samples collected from different areas of Qazvin city, Iran. Contamination with *E. coli* and *C. sakazakii* ordinarily occurs in poor hygienic condition of manufacturing process or failure in thermal process (Saeedi et al., 2017) considering significant correlation between contamination patterns clustered in one group (Fig. 6). Detection of *rfbE* (O157) gene showed that isolated *E. coli* strains were characterized as serotype O157 categorized in Enterohemorrhagic *E. coli* (EHEC) pathotype having the potential of making gastrointestinal disease and hemorrhagic uremic syndrome (HUS). It is worthwhile pointing that the source and reservoir of *E. coli* serotype O157 is animal and associated products which are not using in *Baghlava* as raw materials; consequently, probable cross-contamination after production should be occurred to explain the presence of this serotype (Heiman et al., 2015). Also, Davidson et al. (2015) detected *E. coli* serotype O157: H7 in prevalence investigation of some foodborne pathogens in walnut samples as a non-animal-derived food product (Davidson et al., 2015). Outer membrane protein (*ompA*) gene was detected in *C. sakazakii* strains isolated from our samples characterised for invasion of gastrointestinal epithelial cells and passing through blood-brain barrier leading to diarrheal symptoms and meningitis. Presence of this gene as an important virulence factor of *C. sakazakii*

showed the strength of pathogenicity in our isolated strains (Singh et al., 2017). Usually prevalence of *E. coli* serotype O157: H7 and *C. sakazakii* are evaluated by detection of *rfbE* and *ompA* genes respectively from food samples by researchers (Li et al., 2016).

Prevalence of *S. aureus* and *C. perfringens* as gram positive foodborne pathogens were confirmed in *Baghlava* samples. Because of shape formation and somewhat manufacturing with hands, *Baghlava* products have the potential of contamination with *S. aureus* (Mahmoodi Sadr et al., 2019); although, we detected it in our samples. Isolation of *S. aureus* is quite common in bacterial evaluation of traditional food products because of manufacturing process by hands as reported previously by many researchers in sweet, confectionary and other traditional food products (Demirci et al., 2017). Presence of *S. aureus* was confirmed by primer-specific detection of *nuc* gene encoding the thermostable nuclease of *S. aureus*. With regard to presence of sugar as a dry powder ingredient in *Baghlava* formulation and heat stability of *C. perfringens*, prevalence of this pathogen in this sweet product is probable as it was detected and identified in our samples; however, transmission of this pathogen to traditional products is likely by food handlers (Lee, 2016). Isolation and identification of *C. perfringens* from many traditional foods such as dried beans and rice products have been implemented (Wang et al., 2016). Because of spore-forming characteristic of this pathogen, it can survive in a wide range of processed and dried foods (Luo et al., 2017). Phospholipase C (*plc*) gene encoding alpha-toxin as the main virulence factor belonging to pathogenic *C. perfringens* usually is used for detection and identification of this pathogen in food samples (Hernández et al., 2017). As we identified *plc* gene in our isolates, pathogenic potential of the isolated *C. perfringens* from our samples is discernible. It is needed for more investigation of other virulence factors encoded genes in these isolates because of transmission risk of opportunistic and nosocomial isolated pathogens to human by consumption of contaminated *Baghlava* sweet products. However, using hygienic manufacturing process is necessary to reduce the spreading probability of these opportunistic pathogens from food to consumers leading to decrease the human health threat potential (Paudyal et al., 2017).

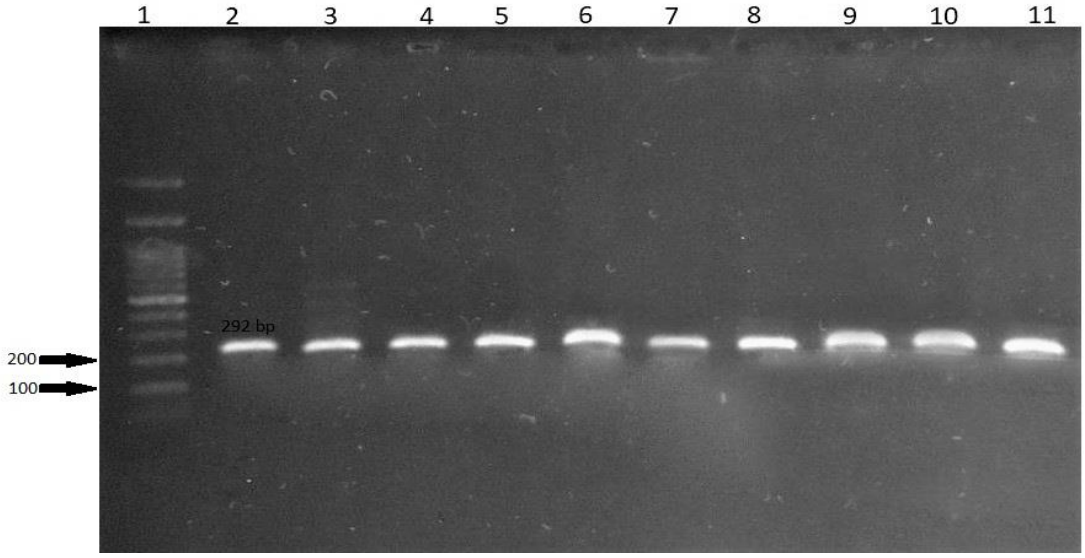


Figure 1. PCR amplification of *rfbE* gene and *E. coli* identification including 100-bp marker (lane 1) and positive samples (lanes 2-11)

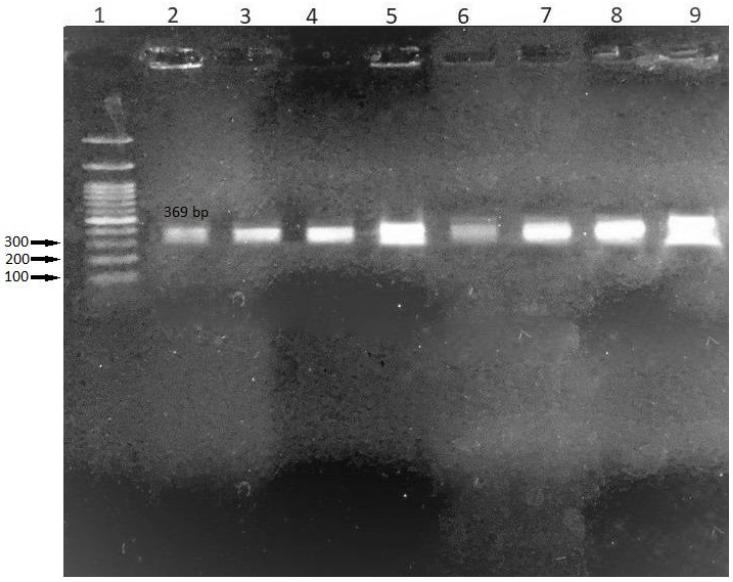


Figure 2. Identification of *C. sakazakii* by PCR amplification of *ompA* gene, lane 1 is 100-bp marker and lanes 2-9 are positive samples

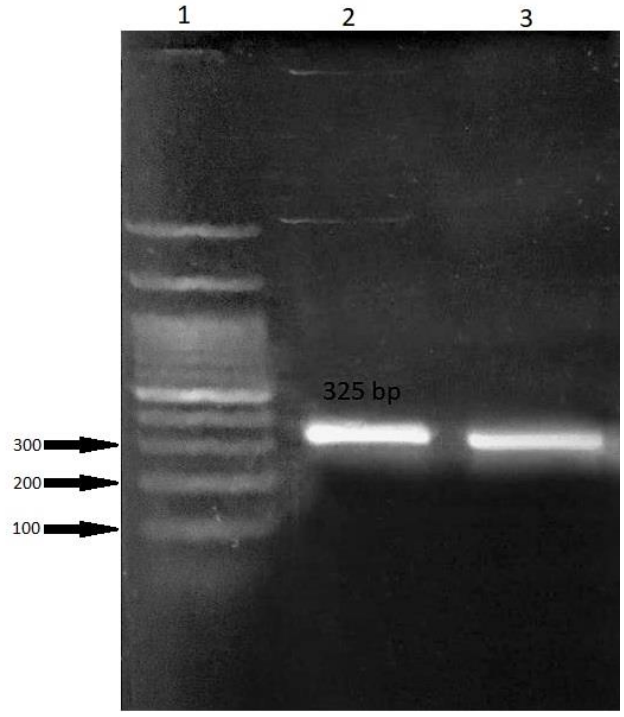


Figure 3. Detection of *plc* gene for confirmation of presumptive *C. perfringens* isolates by PCR assay, lane 1 is 100-bp marker and other lanes are positive samples

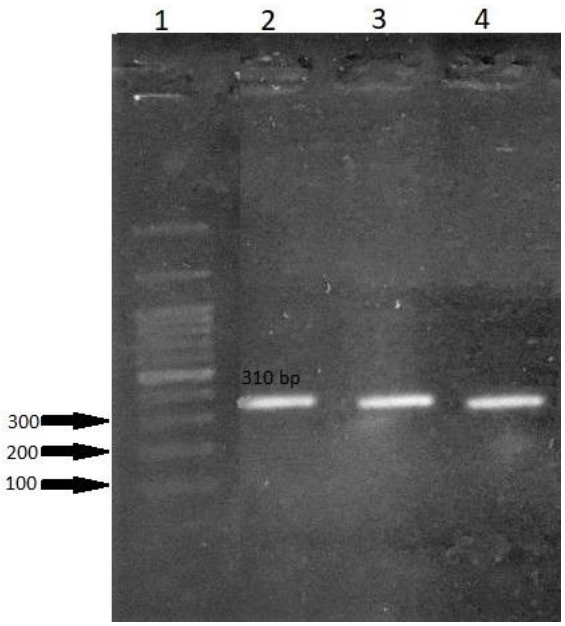


Figure 4.PCR amplification of *nuc* gene for identification of pathogenic *S. aureus* in *Baghlava* samples consisting of lane 1 as 100-bp marker and lanes 2-4 as positive samples

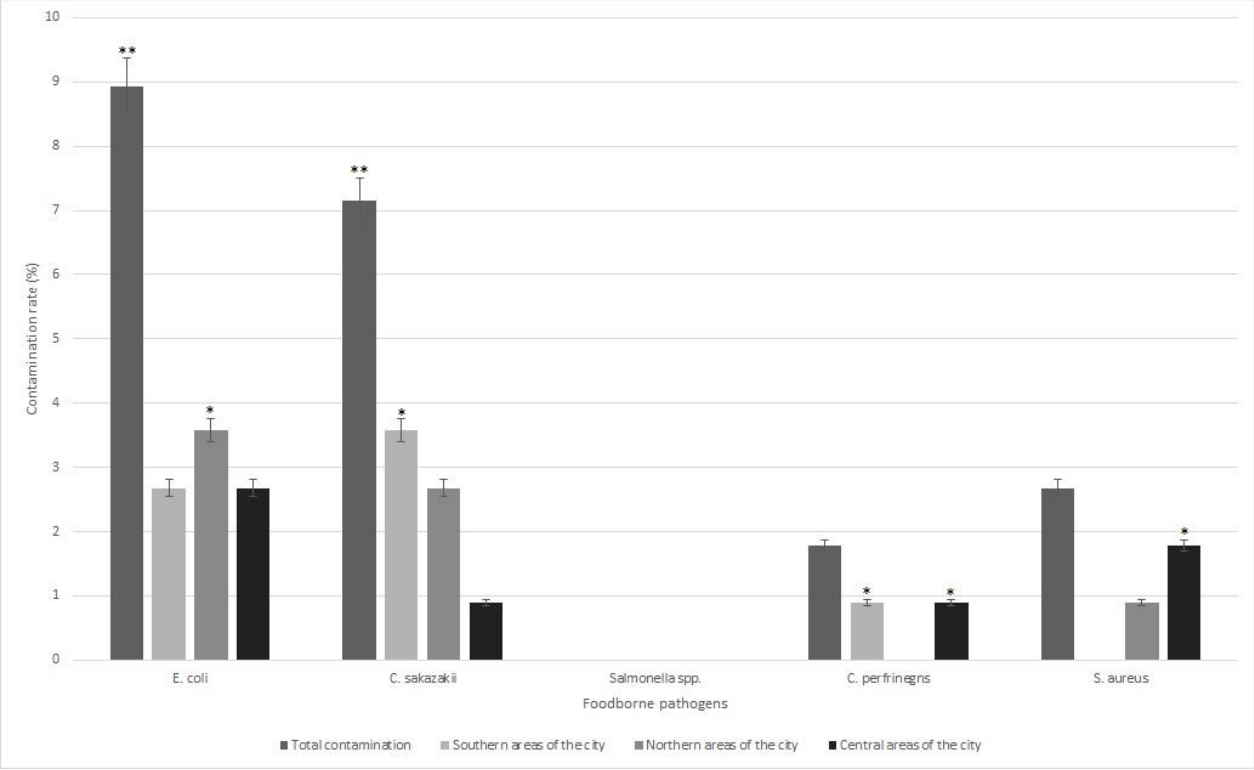


Figure 5. Contamination rate (%) of *E. coli*, *C. sakazakii*, *Salmonella spp.*, *C. perfringens* and *S. aureus* by PCR assay including strains isolated from *Baghlava* samples collected from southern, northern and central regions of the Qazvin city, Iran.

** and * indicate statistically significant differences ($P < 0.05$) between results of total contamination rate and contamination rates in areas of the city for each pathogen separately based on Fisher`s exact test.

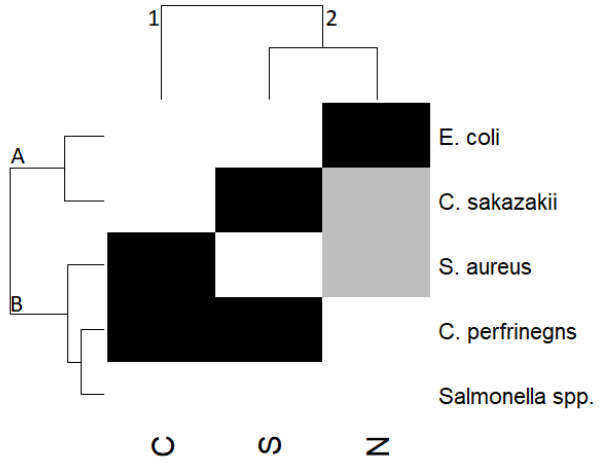
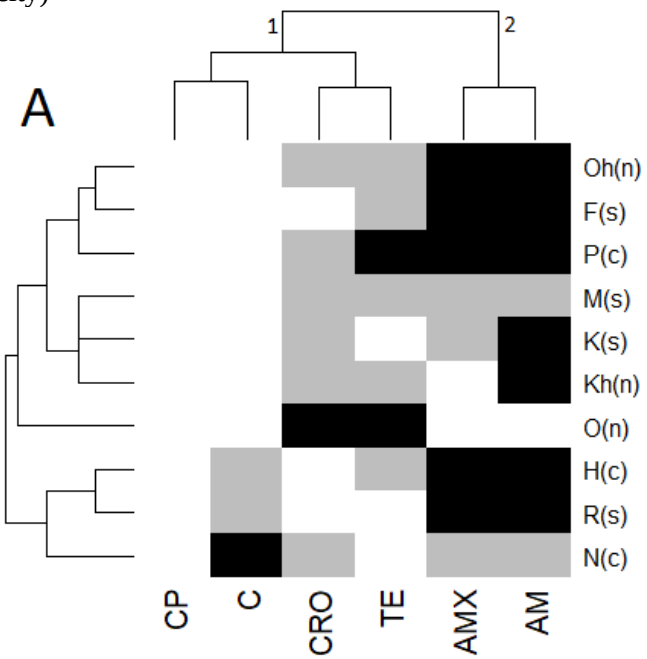
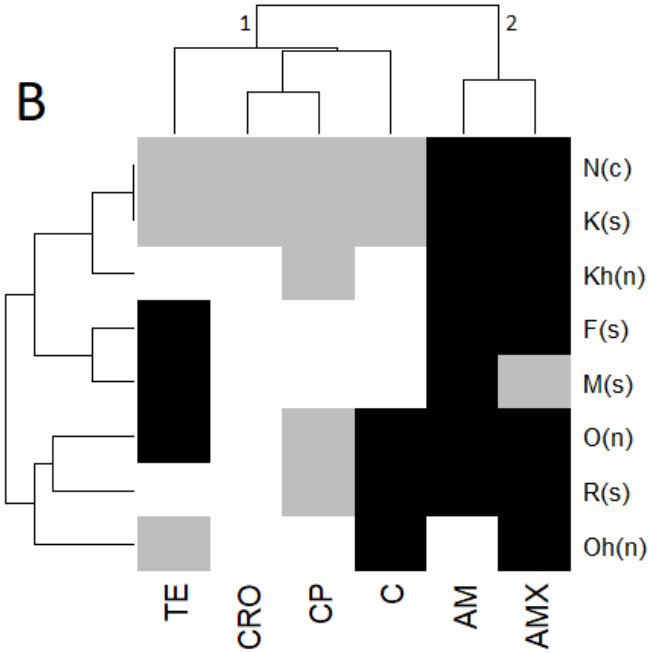


Figure 6. Hierarchical clustering (groups 1-2 for sampling locations and A-B for foodborne pathogens) for contamination rate and prevalence patterns of pathogen strains (White, grey and black colors as low, medium and high contamination rate respectively; rows as isolated pathogens and columns as regions of the sample collection) isolated from *Baghlava* samples collected from different areas of Qazvin, Iran (southern (S), northern (N) and central (C) areas of the city)

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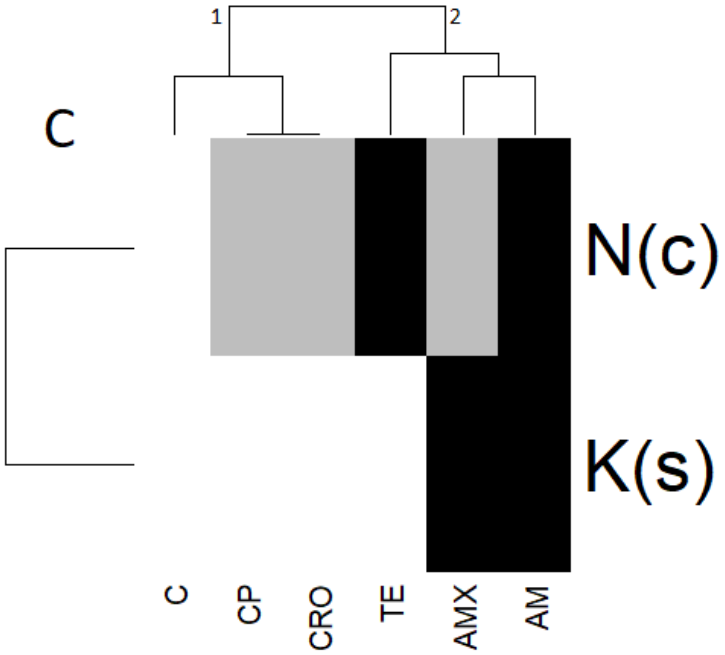
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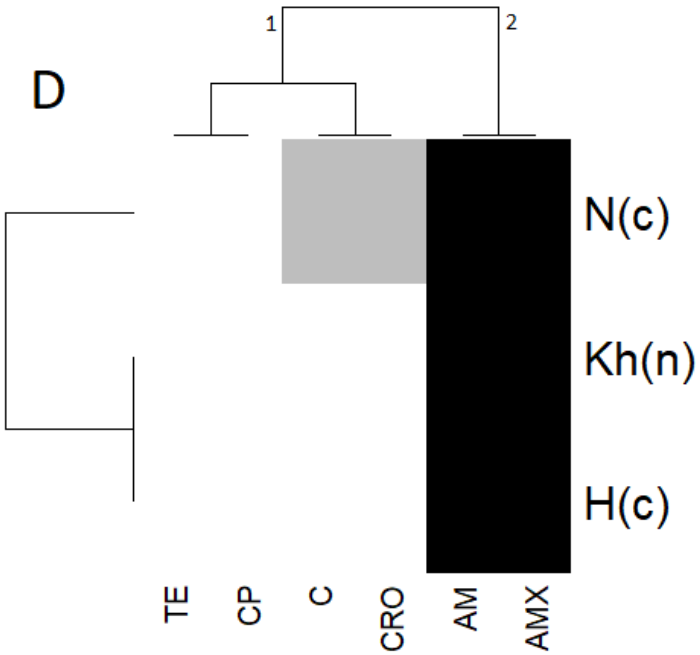

Figure 7. Heatmap and hierarchical clustering of antimicrobial susceptibility phenotypic characteristics of *E. coli* (A), *C. sakazakii* (B), *C. perfringens* (C) and *S. aureus* (D) strains (White, grey and black colors as susceptible, intermediate and resistant to antibiotics respectively; rows as name of the samples and columns as antibiotics) isolated from *Baghlava* samples collected from different areas of the Qazvin, Iran (southern (S), northern (N) and central (C) areas of the city)

3.2. Antimicrobial susceptibility of the isolated strains

Figure 7 A-D provide hierarchical clustering and heatmap of antimicrobial susceptibility properties of the detected pathogens isolated from *Baghlava* samples including *E. coli* (A), *C. sakazakii* (B), *C. perfringens* (C) and *S. aureus* (D). Figure 7 A reveal that isolated *E. coli* strains were more resistant to amoxicillin and ampicillin than other antibiotics visibly illustrated with hierarchical clustering in a significant different group. However, all *E. coli* strains were observed susceptible to ciprofloxacin. Antibiotic resistance pattern of isolated *C. sakazakii* strains (Fig. 7 B) showed strong resistance to amoxicillin and ampicillin as they were categorized in significant different cluster.

On the other hand, susceptibility to ceftriaxone can be distinguished higher than other antibiotics through the AMR patterns of C. sakzakii. Considering low positive samples, most resistance properties for isolated *C. perfringens* and *S. aureus* strains (Fig. 7 C and D) were also observed for amoxicillin and ampicillin antibiotics clustering in the different and significant group as arose from result of gram-negative pathogen AMR patterns. It is worthwhile to note that, susceptibility to chloramphenicol; and tetracycline and ciprofloxacin were detected for *C. perfringens* and *S. aureus* isolates respectively. It is important emphasising that there is not any reasonable relationship between hierarchical clustering of sample types (collected from southern, northern and central areas) in AMR patterns of isolated pathogens. As can be seen in all AMR pattern figures (Fig. 7 A-D), simultaneous presence of amoxicillin and ampicillin AMR phenotypic properties were detected in all gram positive and negative isolated foodborne pathogens according to the results obtained from hierarchical clustering.

Antimicrobial susceptibility profiles of isolates showed resistance to ampicillin and amoxicillin for all isolated foodborne pathogens in this

study, including both gram positive and negative bacteria (Fig. 7 A-D). Several researchers have found phenotypic properties of amoxicillin and ampicillin resistance in bacterial pathogens isolated from food samples (Economou & Gousia, 2015). Ampicillin and amoxicillin resistance properties usually observed in beta-lactamase resistant Enterobacteriaceae family (Bryce et al., 2016) as they were detected in *E. coli* and *C. sakazakii* strains isolated from our samples. Should be considered as multidrug resistant foodborne pathogens, all isolated gram-negative pathogens were resistance to more than one antibiotic illustrated in Fig. 7. However, wide range of antibiotics should be tested on isolated gram-positive pathogens for evaluation of multidrug resistance characteristics (Matuschek et al., 2018). Several researchers showed multidrug resistance properties and complete or intermediate susceptibility to ciprofloxacin for pathogens isolated from food samples (Dan et al., 2015); however, the same results were observed at the present study for all strains isolated from *Baghlava* samples. Multidrug resistance properties of *E. coli* and *C. sakazakii* isolates indicate that antibiotic therapy would not generally be effective for treatment of intestinal and extraintestinal infections and diseases such as bloody-diarrhoea, HUS and meningitis caused by these pathogens (Turnidge, 2015). Also, these results revealed that use of antibiotics must be restricted in agriculture, human and animal health care in Iran.

4. Conclusions

(In the present study, the prevalence of foodborne bacterial pathogens and antibiotic resistance pattern of the strains isolated from *Baghlava* samples manufactured in Qazvin, Iran have been investigated for the first time. Presence of all pathogens except *Salmonella* spp. were confirmed in samples. Prevalence percent of isolated and identified *E. coli, C. sakazakii, C. perfringens* and *S. aureus* strains were 8.92, 7.14, 1.78 and 2.67%, respectively. For each pathogen, most virulence factor encoded gene was detected by PCR assay and the specific primers. All isolates were resistance to amoxicillin and ampicillin considering multidrug resistant foodborne pathogen isolated from *Baghlava*.

On the other hand, based on phenotypic properties of antimicrobial susceptibility testing, all isolated strains were completely and intermediately sensitive to ciprofloxacin. The contamination rate of all pathogens was observed significantly higher in samples collected from southern and northern areas of the city than central region because of more inspection of manufacturers located in central areas of the city by the health monitoring systems. For more safe and higher microbial quality production of *Baghlava*, hygienic manufacturing process and multidrug resistant bacterial evaluation of raw ingredients are suggested.

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