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A recent situation for the presence of *Shigella* spp in local stored cheese in Al-Diwaniyah City, Iraq: *ipaH* and *uidA* virulence genes as molecular targets

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ABSTRACT

The present work, here, was carried out to evaluate the recent situation for the presence of *Shigella* spp. in local stored cheese in Al-Diwaniyah City, Iraq, using *ipaH* and *uidA* virulence genes as molecular targets. The study included the bacterial cultivation of 450 local cheese samples (collected during June to September, 2022) utilizing traditional methodology. The recovered *Shigella* spp. isolates were subjected to a real-time PCR (RT-PCR) that targeted *ipaH* and *uidA* genes for understanding the virulence status of these isolates. The results of the cultivation revealed the presence of 110 (24.4%) *Shigella* spp. isolates in the examined cheese samples. The RT-PCR showed that the virulence genes, *ipaH* and *uidA*, were identified in 84 (76.4%) and 98 (89.1%) of the bacterial isolates, which indicated the presence of *Shigella dysenteriae*. The current findings indicate the current situation of the presence of the virulence *Shigella* spp.

bacteria in local cheese that may need urgent control to eliminate the pathogenic or contamination sources.

Keywords: Dairy products, food hygiene, food poisoning, *Shigella* spp

Introduction

Both wealthy and underdeveloped nations face the challenge of food-borne illnesses. Up to 30% of the globe's community is affected by food-borne illnesses annually, according to the World Health Organization (WHO), while up to 2 million people lose their lives each year. There is a greater severity of the issue in developing countries owing to a deficiency of individual sanitation, food safety procedures, and accurate data on food borne infections due to inadequate or non-existent monitoring standards [1,2].

Food-borne illnesses pose a significant risk to world population, leading to a heavy public health impact and substantial financial consequences. Latest numbers from the WHO put the annual mortality toll in some countries, such as nations from Africa to reach up to 700,000 fatalities. Sporadic instances, which make up the bulk of the problem, remained unreported, thus these outbreaks only represent what's visible at the surface [3,4].

Most cases of shigellosis occur in the world's poorest, most underdeveloped regions. This is because these regions generally lack access to clean water and sanitation facilities. This suggests that Shigellosis is a major public health issue, where poor sanitation and contaminated water are common [5,6]

Because of to their fecal-oral transmission technique and minimal pathogenic dosages, Shigella spp. are a significant cause of foodborne illnesses. Overpopulation, lack of access to clean water and hygiene practices, improper disposal of human waste, and improper disposal of waste from food preparation all contribute to the spread of disease. Globally, contaminated food and water are responsible for between 3 and 5 billion incidences of infectious diarrhea and 1.8 million fatalities per year, most of which are in infants and young children [7,8].

The present work, here, was carried out to evaluate the recent situation for the presence of *Shigella* spp. in local stored cheese in Al-Diwaniyah City, Iraq, using *ipaH* and *uidA* virulence genes as molecular targets.

Materials and methods

Samples

The study included the bacterial cultivation of 450 local cheese samples collected during June to September, 2022, from local stores in Al-Diwaniyah City. Each sample at 25gm was inserted in a 225ml of 0.1% peptone and mixed-homogenized with 3mg/ml novobiocin was added to the mixture to prevent the growth of other bacterial organisms. For enrichment purposes, 1ml of the mixture was added to 9ml of broth and incubated at 37˚C for 18hrs. Then, McConkey and *Salmonella-Shigella* agars were employed to recover *Shigella* spp. TSI and IMViC were recruited for the confirmation of the bacterial identity.

Shigella-DNA extraction

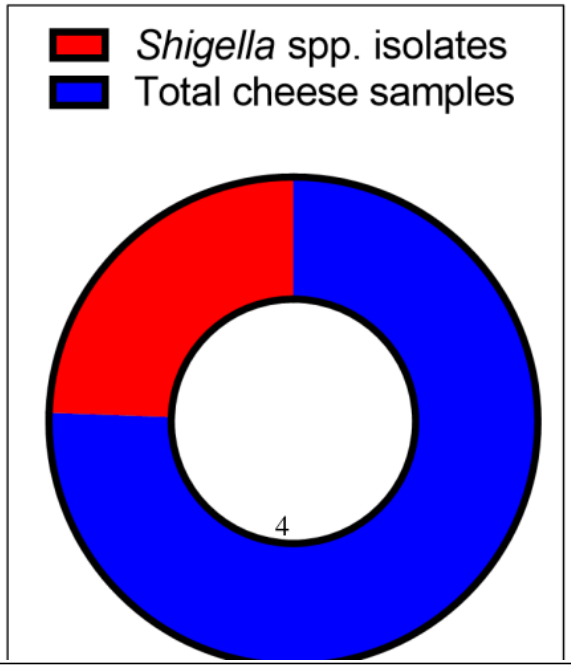
The PrestoTM Mini gDNA kit (Geneaid, USA) and its protocol steps were followed to extract the *Shigella* DNA. A bacterial enrichment step on a one-ml BHI broth for overnight was completed. Then, after a 10000rpm-60s-centrifugation step, the precipitant was collected to extract the DNA materials. A NanoDrop was employed to evaluate the purity and concentrations of the material.

ipaH and *uidA* dependent RT-PCR

The recovered *Shigella* spp. isolates were subjected to a RT-PCR that targeted *ipaH* and *uidA* genes for understanding the virulence status of these isolates and confirming the identity of the bacterial species. The primers, F: TTTCGCTGTTGCTGCTGATG and R: TCGAAAAGGCCTTCTGATGC, and F: TTGCGCAAGACTGTAACCAC and R: AGTTCAACGCTGACATCACC, respectively, were designed utilizing Primer3 Plus and NCBI-based website tools, and placed in the relevant database under the accession numbers of KR269602.1 and AY698483.1, respectively. The AccuPower® GreenStarTM qPCR PreMix kit (Geneaid, Korea) was followed to perform the RT-PCR. For 20μl of a total volume, 5μl *Shigella* DNA, 1μl (10pmol) of each primer (F or R), and 13μl molecular-use water, were mixed together and were added to other components. The runs of a thermocycler were performed under one-cycle of 3mins-95˚C Initial denaturation, 45 cycles of each of 10s-95˚C denaturation, 30s-60˚C annealing, and 30s-60˚C detection, and one-cycle of 0.5s-60˚C to 95˚C melting.

Results

The results of the cultivation revealed the presence of 110 (24.4%) *Shigella* spp. isolates in the examined cheese samples (Figure 1). The RT-PCR showed that the virulence genes, *ipaH* and *uidA*, were identified in 84 (76.4%) and 98 (89.1%) of the bacterial isolates, which indicated the presence of *Shigella dysenteriae* (Figure 2).



have reported shigellosis outbreaks linked to milk and milk products in underdeveloped nations [9-11].

Of the Shigella species (*S. dysenteriae, S. flexneri, and S. sonnei*) that were isolated, *S. dysenteriae* was the most common, as documented by Elkenany et al [12]. A total of 71.4% of Shigella organisms tested positive for resistance to multidrug.

Further research indicated that *S. dysenteriae* is the most frequent species of *Shigella*, despite claims that *S. flexneri* is the primary cause of shigellosis in undeveloped nations [13]. Ahmed and Shimamoto [14] found that Shigella spp. were present in 1.4% of dairy products, with *S. flexneri* predominating. Eight-point seven percent *S. flexneri* was found in milk products by Tambekar and Bhutda [15]. Market-bought cheese was shown to have a greater prevalence of *Shigella* spp. Contamination [12].

Inadequate sanitary practices while milking, manufacturing, packaging, and transportation of milk and milk products could increase the bacterial contamination by this bacterium. Contaminated water and feces are the principal source of Shigella and may account for the high incidence of Shigella in our research. Hence, efficient animal health management, effective sanitation and disinfection methods of the milking equipment, clean water, and personnel hygiene are essential in animal farms to reduce the danger of Shigella spreading to other animals and humans [16].

Conclusion

The current findings indicate the current situation of the presence of the virulence *Shigella* spp. bacteria in local cheese that may need urgent control to eliminate the pathogenic or contamination sources.

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