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Original Research Article

The Current Prevalence of the Food-Borne *Shigella* Spp in Local Stored Cheese in Al-Diwaniyah City, Iraq: The Virulence *ipaH* and *uidA* Genes as Targets for a Molecular Study

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Abstract: The current study was carried out to evaluate the current prevalence of the food-borne pathogen; *Shigella* spp., in local stored cheese in Al-Diwaniyah City, Iraq, by using the virulence *ipaH* and *uidA* genes as targets for molecular tools. The study included the bacterial cultivation of 450 local cheese samples (collected during June to September, 2022) utilizing conventional methodology. The recovered *Shigella* spp. isolates were subjected to a real-time PCR (RT-PCR) that focused on the genes mentioned above. 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 were identified in 84 (76.4%) and 98 (89.1%), respectively, in the bacterial isolates, which indicated the existence of *Shigella dysenteriae*. The current findings indicate the presence of *Shigella* spp. in local cheese, which needs urgent control to eliminate the source of infection or contamination.

Keywords: Food-borne, food hygiene, food poisoning, *Shigella* spp.

INTRODUCTION

Shigella is the causative agent of shigellosis, one of the major diarrheal diseases in humans. Like the other members of Enterobacteriaceae, Shigella can cause mild watery or severe dysenteric diarrhea. The severity of the disease, which can lead to death due to dehydration, malnutrition, and weight loss, depends on gender, age, and immune status, especially malnourished children and sexually active young persons. According to a WHO report, the highest incidence of cases was found in children aged 1 to 5 years with around 27% and 21% from 1 to 14 percent of cases among 1–4 and 5–9 years of children, respectively. However, in recent times, shigellosis has spread to tourists and indigenous people in developed nations due to globalization and international travel [1]. Due to the global spread of shigellosis and most shigellae are resistant to common antibiotics, EWS is necessary for this pathogen to reduce the impact on the community 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 [2].

Food-borne illnesses pose a significant risk to world population, leading to a heavy public health impact and substantial financial consequences. WHO figures from late 2012 suggest an annual death toll of up to 700,000 in countries from Africa alone? Sporadic outbreaks (which is where the real trouble lies) were not reported. All these outbreaks show only the tip of the iceberg [3,4].

It is, however, quite probable that our sample population is also referring more generally to shigellosis cases worldwide, in what is clearly the poor, underdeveloped part of the world: regions where access

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to clean water and sanitation facilities are the last things on – or have been for a long time – the menu [5]. Most cases of shigellosis worldwide are contracted in the poorest, underdeveloped world, generally missing out on clean water and sanitation facilities. This might well mean that Shigellosis equals a huge problem in public health, where the poor sanitation and polluted water are part and parcel of the lives of most of the people [6].

Owing to their fecal-oral transmission mode and low infectious doses, as well as the gastrointestinal disease they cause, Shigella spp. are a frequent cause of foodborne illness. Overcrowding, lack of clean water and hand hygiene, poor disposal of human feces, and poor disposal of feces from food preparation are associated with disease. Globally, food and water contamination accounts for 3 to 5 billion episodes of infectious diarrhea and 1.8 million deaths each year, mainly in children under [7,8].

This work was undertaken to detect presence of Shigella spp. in local stored cheese in Al-Diwaniyah City, Iraq using ipaH and uidA as target virulence genes.

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).

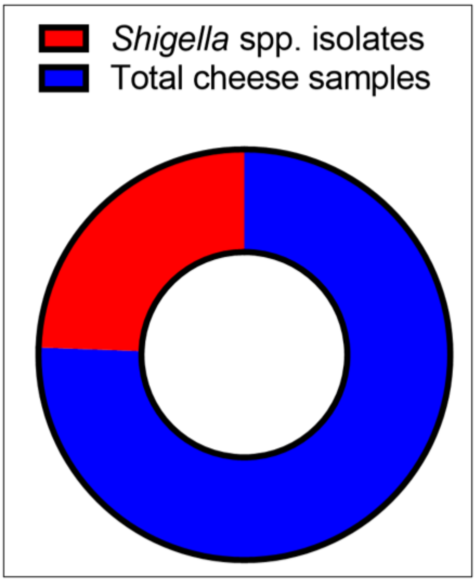

Figure 1: Total number of *Shigella* spp. isolates (110) out of total number of cheese samples (450)

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).

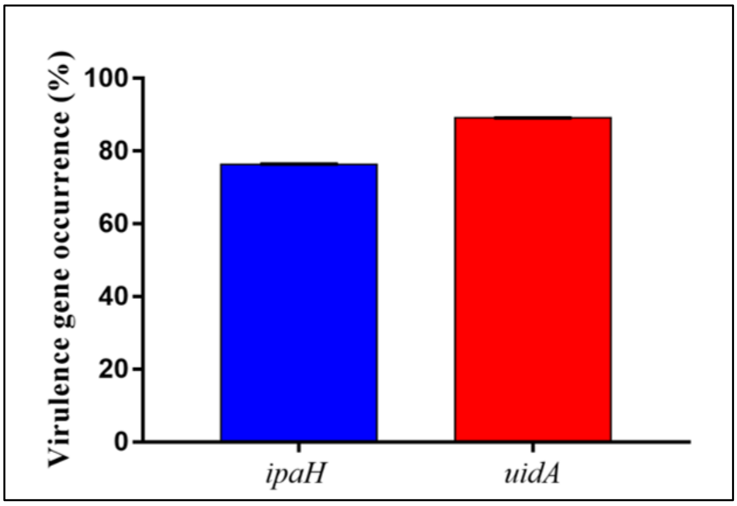

Figure 2: Incidence rates of *Shigella dysenteriae* virulence genes in local cheese samples

DISCUSSION

In low-resource settings, shigellosis is a significant public health issue since it is one of the most common acute gastrointestinal infections. In this case, it's the gram-negative Enterobacteriaceae bacteria that are to blame. Despite the importance of milk and milk products as vectors for the spread of food-borne diseases to people, few publications 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].

Shigellae still rank at the top among diarrheal pathogens in terms of morbidity and burden. The Shigella genus is small, but it has a big impact on human health. Every year, 175 million cases of Shigella infections are reported worldwide. Two-thirds of these cases occurred in developing countries and caused 450 thousand deaths, mostly in children under five years old. Along with enteroinvasive E. coli (EIEC), Shigellae are the primary cause of bloody diarrheal disease in humans. Vaccination would offer a solution to the problem, but a safe and effective vaccine is yet to be licensed. In addressing the problem, researchers have been studying the physiology, the pathogenic mechanism, pathogen-host interactions, and successful interventions. Contemporary studies utilized in vitro epidemics, in vivo animal models, and human challenge studies. Great achievements in deciphering Shigellae biology still await a better mimic of the human infection [17, 18].

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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Conflict of Interest: There is no conflict of interest.

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Availability of Data and Material

The data presented in this study are available on request from the corresponding author.

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