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Molecular identification of Pathogenic Microbes in some primary schools' canteens in Jeddah Province

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Contaminated food contributes to significant public health issues across the globe. It is caused by toxins produced by microorganisms that contaminate food products, including pathogenic bacteria, viruses, and toxins. Every school year starts, and many possible risks and worries accompany it. As children come into contact with various cuisines at school or consume contaminated lunches, we may observe a rise in food poisoning events and an increase in cold and flu cases. Pathogenic microorganisms pose significant risks in schools due to frequent contact with various microorganisms throughout the day. The canteens are prime locations for microorganism transmission to young children. This study aimed to investigate the occurrence of bacteria in samples taken from various areas within primary school canteens. The study aimed to determine whether these canteens are adequately sanitized and clean for children or if they serve as the main source of their illnesses. The study was conducted in five private and five government schools to isolate various bacterial strains and identify pathogenic microbes in the Primary Schools' Canteens in Jeddah Region, Saudi Arabia, using microbial and molecular identification techniques. From 100 samples collected from various surfaces in the canteens, a total of 161 bacterial isolates were obtained, and a subset of 42 isolates with distinct morphologies was selected for further analysis. The study observed that the amount of bacteria in private schools was higher than in government schools. Among the ten canteens examined, Bacillus species accounted for an average of 33.3% of the total bacterial strains found, followed by 26.19% for Staphylococcus species, with other strains ranging from 2.3% to 9.5%. The study also observed variations in the size of the inhibition zone, ranging from 10 mm to 40 mm, indicating differences in bacterial susceptibility to the tested antibiotics. Furthermore, a significant number of isolates exhibited resistance to multiple antibiotics, suggesting the presence of multi-antibiotic resistance. Regarding the hemolytic activity of the isolates, the study found that 33.3% exhibited β-hemolysis, while 26.9% showed moderate hemolytic activity (α-hemolysis). In contrast, no hemolytic activity (γ-hemolysis) was observed in 40.47% of the isolates. In addition, the bacterial isolates were subjected to a disk diffusion test to assess their susceptibility to a commercial disinfectant commonly used for school surface cleaning called "Safe." The results indicated that there was no inhibition of bacterial growth at a 1% concentration of Safe. However, as the disinfectant concentration increased, the inhibition zone expanded. At a 10% concentration, the inhibition zone measured 9.76 mm, progressively increasing to 13.82 mm, 19.6 mm, and 28.25 mm at 25%, 50%, and 100%, respectively. This study concludes that various bacteria contaminate all the surfaces analyzed in ten schools. The results indicate that public and private school students are at a high risk of contracting significant bacterial infections due to the abundance of microorganisms in the school environment. It emphasizes the need to develop new approaches to improve hygiene in schools, making them healthier and safer environments for learning.

Keywords: School, Canteens, Students, Molecular, Disinfectants, Bacteria.

INTRODUCTION

Contaminated food contributes to significant public health issues across the globe. It is caused by toxins produced by microorganisms that contaminate food products, including pathogenic bacteria, viruses, and toxins(Pandey, 2016). In 2010, the World Health Organization (WHO) predicted that 600 million people worldwide were infected with food-borne diseases (FBDs), resulting in 420,000 deaths (Devleesschauwer *et al*. 2018; Yemane and Tamene 2022).According to

these statistics, 7.69% of the world's population gets FBDs annually, and 7.5% of all fatalities are caused by food-borne illnesses(Yoon and Chung 2018).Around 100 million cases of FBDs are thought to occur annually in the Eastern Mediterranean Region (EMR), making it the third-highest region for FBDs(Organization 2015; Alhashim *et al*. 2022).Around 1647 food-borne illnesses were reported in 2010, according to a document published in 2013 by the Health Ministry of the Kingdom of Saudi Arabia. According to another report, 255 documented cases of food-borne illnesses in 2011, which sickened 2066 persons (Al-Shabib *et al*. 2017). A recentstudy reported that Saudi Arabia had 3081 FBD cases in 2020, with 1258 of those instances occurring in people between the ages of 15 and 45 (Faour-Klingbeil *et al*. 2022). Foodborne infections have spread rapidly in Saudi Arabia over the past 30 years due to the country's fast socioeconomic development and urbanization (Alshanbari 2022). More incidences of foodborne disease (FBDs) outbreaks from schools are reported worldwide, including in Saudi Arabia(Ababio 2015; Al-Mohaithef 2021). According to previous studies, bacterial agents such as *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., Escherichiacoli sp, *Listeria* spp., and *Vibrio* spp and others have been implicated in foodborne diseases (El-Sheikha 2015).

Every school year starts, and many possible risks and worries accompany it. As children come into contact with various cuisines at school or consume contaminated lunches brought from home, we may observe a rise in food poisoning events in addition to an increase in cold and flu cases. In Japan, 11,826 cases of *E. coli* 0157: H7 infection were reported in schools in seven months in 1996, with 12 deaths, whereas in Brazil, 11.6 % of verified FBD in 2005 came from school food services (Ababio *et al*. 2016; Santana *et al*. 2009). In France, 544 teenagers got Salmonella food poisoning in 2010, while in Germany, 11,200 pupils from hundreds of schools were infected with norovirus in 2012(Marzano and Balzaretti 2013). To our knowledge, few studies have associated food sources, drinking water, personal hygiene, and practices with food-borne diseases in schools in Saudi Arabia (Al-Ghamdi *et al*. 2009). Fareid *., et al*.,(2011) isolated about sixpathogenic bacterial isolates from School Children in Hail, Saudi Arabia. These bacteria were Salmonella spp., *Escherichiacoli*, *Shigella*spp., *Aeromonas* spp., *Proteus* spp., and *Klebsiella* species. Faried illustrated the occurrence of the different bacterial pathogens. *Escherichia coli* were the most commonly isolated organisms, followed by *Klebsiella* spp. and *Shigella* spp., *Proteus* spp. was less commonly isolated (Fareid *et al*. 2011). Pu (2021), investigated the epidemiological characteristics of school foodborne diseases in Henan Province from 2011 to 2020 to lay the groundwork for effective outbreak prevention and management. They found that in the last ten years, 47 outbreaks of school foodborne infections have been documented in Henan province, resulting in 1258 cases, 701 hospitalizations, and one fatality. The months of June and September saw the highest number of foodborne disease incidences in schools(Pu*et al.,*2021).

The majority of occurrences occurred in middle school and primary school canteens. The major pathogenic agents that caused outbreaks of foodborne diseases in schools were pathogenic bacteria and their toxins, accounting for 78.26% of the identified causes. *Bacillus cereus* was the most common pathogen responsible for outbreaks of foodborne illnesses in schools. Diarrheagenic *Escherichia Coli* was the pathogenic factor that produced the most cases. Apart from inexplicable events, foodborne disease outbreaks in schools were primarily caused by faulty handling. Conclusion Foodborne disease outbreaks in schools are most common among primary and middle school children (Pu *et al*. 2021). Bankolé (2012) conducted a study investigating the spread of microorganisms that cause foodborne illness among school canteen employees. Three hundred thirty-sixsamples were taken from the nose, mouth, and hands. He discovered that the vendors had various dangerous bacterial strains, including *Staphylococcus aureus* (26/122), sulfate-reducing clostridia (14/122), and *Escherichiacoli* (10/122). According to the findings, food vendors at the school canteen may be the carriers of bacteria that cause children's food poisoning (Bankolé et al. 2012) . Food poisoning is more common in children who eat from school canteens rather than bringing packed food from home. Is the school canteen providing hygienic, fresh food? This is certainly a concern for most parents. Meftahuddin (2002) states that the reported incidence rates of major food-related diseases have gradually reduced from 1988 to 1997 except for food poisoning and cholera. A study of food poisoning outbreaks between 1996 and 1997 discovered that 66.5 % of outbreaks occurred in schools.

In contrast, just 0.4 % were caused by tainted food sold at numerous public food outlets (Meftahuddin 2002). Food handler hygiene is one of the factors that contribute to the occurrence of foodborne infections, such as food handlers with poor personal hygiene who have had typhoid and who do not wash their hands properly. Hedican conducted a study of food handlers in a restaurant in 2009 and found that Salmonella contamination testing was positive among them (Susanna *et al*. 2020).

In Saudi Arabia, El-Sheikh (2001), reported that various enteropathogens among children in Jeddah city were *Escherichia coli*, including enterohaemorrhagic *E. coli* and enteropathogenic *E. coli* (EPEC). Other detected bacterial pathogens were: *Klebsiellapneumoniae*, *Salmonella* sp., *Shigellaflexneri*(El-Sheikh and El-Assouli 2001).*Salmonella* and *Shigella* species were found to be

bacterial enteropathogens in young Saudi Arabian children with acute diarrhea, according to Johargy(2010)(Johargy et al. 2010). The Ministry of Health (MOH) of Saudi Arabia and the local food sector are both aware of the dangers that food-borne diseases and infections pose to the Kingdom's people and residents. They are attempting to identify bacteria that cause foodborne illnesses. In light of previous national and international studies (Alhashim et al. 2022; et alAl-Mohaithef 2021; Mohamed et al. 2017; Nam et al. 2023) that investigated the occurrence of pathogenic microbes in schools' canteens. The traditional approach to identifying and detecting a food-borne gastroenteritis outbreak focuses on the culture, counting, and isolation of putative colonies for further identification and analysis. Because microorganisms can quickly modify their phenotypic features in response to environmental changes or genetic mutations, these phenotypic typing approaches also have some limitations.

This study aimed to isolate various bacterial strains and identify pathogenic microbes in the Primary Schools' Canteens located in Jeddah Region, Saudi Arabia, using microbial and molecular identification techniques.

MATERIALS AND METHODS

Study location

The study was conducted in 10 primary schools selected from a total of 1747 schools in the Jeddah governorate, Saudi Arabia. These schools collectively had 528,548 students, with an average of 300 students per school (MOE 2023). Jeddah's weather is characterized as hot and humid, with temperatures between 25 to 40 degrees Celsius, which can foster the growth of microbes, particularly in environments with large numbers of people, such as schools (Alshehri and Saeed 2013). The current study was carried out at the Biological Science Dept., Faculty of Science, at King Abdulaziz University. The experimentation was done during the period 2022-2023.

Microbial Studies

Collection of samples and isolation of bacteria

Collection samples from children between the ages of six to twelve is a critical process that must be approached with care to avoid causing fear or disrupting their freedom. In the current study, surface swab samples were obtained from various locations within school canteens in Jeddah, Saudi Arabia, with a focus on areas with high contact frequency by children, including tables, equipment, food wrapping paper, desks, door handles, banisters, taps, staff hands, drinking water fountains, and hand wash faucets. The experimental protocol was approved, and staff consent was obtained before collecting samples from their hands. Ten sites were selected from each school, and a sterile cotton swab was utilized to collect samples from the desired surface, which were then placed in a tube containing sterile peptone water for transportation. The swabbed area covered approximately 10x10cm, and 10 samples were taken from each school, resulting in a total of 100 sample sites from all schools. The samples were transported to the laboratory in sanitized conditions to prevent contamination, and one ml water samples were collected in test tubes. Immediately upon collection, the samples were cultured, and a series of isolation procedures were performed to identify the types of bacteria present in each sample.

Isolation and Preservation of bacterial isolates

For this study, samples were collected from various locations within ten schools. Each sample, measuring one mL, was transferred to separate petri dishes containing selective, differential, or enriched media such as Blood agar medium (BA), Nutrient agar (NA), MacConkey agar, Mannitol salt agar (MSA), and Salmonella-Shigella agar (SSA) plates. Using a sterile loop, the sample was spread evenly onto the surface of the media. The plates were then incubated overnight at 37°C in aerobic conditions. After the incubation period, individual colonies that had formed were selected and cultivated again on the same media to obtain pure, single colonies. The colonies were differentiated based on their morphological characteristics, including surface texture, elevation, diameter, color, edges, and pigmentation. To ensure the preservation of these distinct colonies, repeated streak culture was performed using the same medium. Finally, the purified bacterial isolates were stored in a 25% glycerol solution at -80°C for future analysis.

Antibiotics susceptibility testing of bacterial isolates.

The antimicrobial profile of the tested bacteria was determined using the Kirby-Bauer disk diffusion method, following the guidelines provided by the Clinical and Laboratory Standards Institute. The antibiotics used in this study included Penicillin G (PG) at a concentration of 10 units, Cephalothin (KF) at 30µg, Erythromycin (E) at 15µg, Ampicillin (AP) at 10µg, Clindamycin (CD) at 2µg, and Cotrimoxazole (TS) at 25µg. These antibiotics were obtained from Oxoid, Basingstoke, UK, following Wayne's instructions (Wany 2008).

Disinfectant Susceptibility Testing of bacterial isolates

A commercial product commonly utilized in schools for cleaning surfaces (Safe) was procured from the local market. The agar-disk diffusion technique was employed to assess the bacterial isolates' susceptibility, and the mean diameters of the zone of inhibition created were calculated. In this method, a bacterial inoculum (equivalent to 0.5 McFarland) was spread over the entire

agar surface, and a 6 mm disk was placed on the surface, followed by applying 100 µL of the disinfectant with different concentrations (1, 10, 50, and 100%). The agar plates were subsequently incubated under appropriate conditions depending on the investigated bacteria.

Hemolytic Activity of Bacteria on blood agar

Hemolytic activity is carried out by growing bacteria on blood agar plates. The plates are then incubated for some time, typically 24-48 hours. After incubation, the plates are examined for the presence of hemolysis. The type of hemolysis is determined by the appearance of the agar around the colonies.

Identification of bacteria

Gram staining

The study involved performing gram staining on different colonies of bacteria for microscopy. Following the method described by Vincent and Humphrey in 1970, the Gram staining technique was utilized to differentiate between Gram-positive and Gram-negative bacteria(VincentandHumphrey,1970). The process involved applying various solutions, such as crystal violet, iodine, ethanol, and safranin, and rinsing with sterilized distilled water between each step. The stained slides were then observed under a light microscope.

Molecular identification of bacterial isolates through 16s rRNA gene sequencing and phylogenetic analysis:

Isolation of chromosomal DNA

In this procedure, a modified version of the method outlined by Azcárate-Peril and Raya in 2001 was used to extract total genomic DNA. Bacterial pellets obtained from overnight cultures were combined with TES buffer and lysozyme, and the mixture was incubated at a temperature of 37°C for a duration of 20 minutes. Subsequently, proteinase K was introduced and further incubated at the same temperature. After transferring the mixture to an ice bath, sodium acetate and chloroform:isoamyl were added, and the resulting mixture was subjected to centrifugation. The upper phase containing the desired DNA was cautiously transferred to a new container, and isopropanol was added, followed by overnight storage at -20°C. The next day, the solution was centrifuged, the liquid phase was discarded, and the DNA was air-dried before being re-suspended in distilled water. To evaluate the quality of the DNA, a 10 µl portion of the isolated DNA was loaded onto a 0.5% agarose gel in 1x TBE buffer and subjected to electrophoresis at 100 V for 60-90 minutes. Finally, the gel was stained with ethidium bromide.

Polymerase chain reaction

The DNA of the isolates was amplified using PCR with the 16S rDNA gene as the target. The amplification process involved using specific primers (27F and 1100R) and a PCR thermal cycler. Each reaction mixture contained a Master-mix with Taq polymerase, dNTPs, and MgCl2, along with ultrapure PCR water, forward primer, and DNA template. The amplification followed standard protocols, including denaturation, annealing, and elongation steps. The success of the amplification was confirmed by observing ethidium bromide fluorescence in a gel electrophoresis assay using agarose gel.

Gel electrophoresis

Agarose gel electrophoresis was used to separate DNA fragments based on their sizes. A gel was prepared by dissolving agarose in TBE buffer, and ethidium bromide was added for visibility. The gel was set in a tray with a comb to create wells for sample loading. After removing the comb, DNA samples mixed with loading dye were loaded into the wells. Electrophoresis was performed, applying a voltage for a specific duration. The separated DNA fragments were then visualized under UV light.

Sequencing analysis of 16s rRNA

The 16S ribosomal RNA gene sequencing was performed by Macrogen Lab in South Korea for bacterial identification. Partial sequences of the gene were obtained using specific primers and amplified through PCR. The obtained sequences were then analyzed using Blast search to find the closest match in the GenBank database, enabling the identification of the bacteria.

Phylogenetic analysis:

The sequences were manually edited using SnapGene Viewer software version 3.3.3. Subsequently, they were compared to the GenBank database of the National Center for Biotechnology Information (NCBI) using the BLASTN search. Reference sequences were obtained from the database for phylogenetic analyses. The phylogenetic trees were constructed using MEGA software, which is accessible on the NCBI website.

RESULTS

Description of the samples

In this study, 100 surface swab samples (10 school canteens) were collected from various locations within school canteens in Jeddah, Saudi Arabia, with a focus on areas with high contact frequency by children, including tables, equipment, food wrapping paper, desks, door handles, banisters, taps, staff hands, drinking water fountains, and hand wash faucets and analyzed during the period from April 2022 to June 2023. These schools are distributed in different areas in the

city of Jeddah. This study included government schools (five schools) , and private schools (five schools), selected from a total of 1747 schools in the Jeddah governorate, Saudi Arabia. These schools collectively had 528,548 students, with an average of 300 students per school.

Collection of samples and isolation of bacteria

In this study, 100 swabs (10 school canteens) were collected and analyzed during Jan 2023. The purification of isolated bacteria is the process of obtaining single colonies of bacteria from a mixed population, the isolates were re-cultured to get a pure single colony. In total, 161 isolates were isolated, of which forty-two isolates had different morphological which were selected for further investigation, and the samples were designated as school 1(F-1), school 2(F-2), school 3(F -3), school 4(F -4), school 5(F-5), school 6 (F -6), school 7(F -7), school 8(F -8), school 9(F -9) and school 10(F-10) shown in Table 1. All isolates were designated as F1 to F161.

The outcomes of this study indicated that private schools had more isolated bacteria than government schools. Specifically, 87 (54.1%) of the 161 isolates were from private schools, while 74 (45.9%) were from government schools (Figure 1). The distribution of bacterial isolates across schools was also found to vary, with Riyad Alquran schools (F.9) showing the highest level of contamination at 17.34% (28/161), followed by Al-Thager Model School Complex (F.4) at 14.28% (23/161), Al Anjal Private School (F.6) at 12.42% (20/161), Imam Abu Amr Al-Basri Primary School (F.5) at 11.18% (18/161), and Prince Sultan Educational Complex (F.1) and Alrahmah Private School (F.8) both at 9.94% (16/161). Jeddah Kindergarten (F.7) had a contamination rate of 8.69% (14/161), while Al-Noor Educational Complex (F.2) had 8.83% (11/161) and Alhejez School (F.3) had 5.59% (9/161). The lowest contamination rate was observed in Abdurrahman bin Auf school (F.10) at 3.72% (6/161) (Figure 2).

Table 1: Numbers of bacteria isolated from various schools' canteens from Jeddah in Saudi Arabia

No	Code	Name of Schools	Type of Schools	No. of isolates	Percentage %
	F-1	Prince Sultan Educational Complex	Governmental	16	9.93
	F-2	Al-Noor Educational Complex	Governmental	11	8.83
	F-3	Alhejez school	Private	9	5.59
	F-4	Al-Thager Model School Complex	Governmental	23	14.28
	F-5	Imam Abu Amr Al-Basri Primary School	Governmental	18	11.18
	F-6	Al Anjal Private School	Private	20	12.42
	F-7	Jeddah Kindergarten	Private	14	8.69
	F-8	Alrahmah Private School	Private	16	9.94
	F-9	Riyad alquran schools	Private	28	17.34
	F-10	Abdurrahman bin Auf school	Governmental	6	3.72

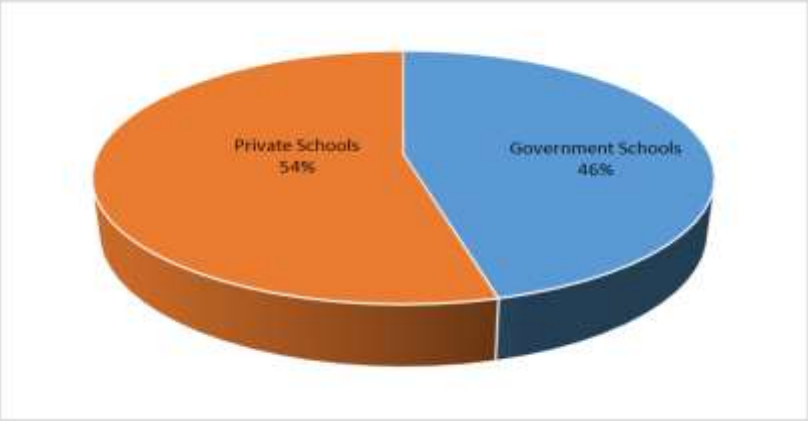

Figure 1: Distribution of bacteria isolated from government and private schools.

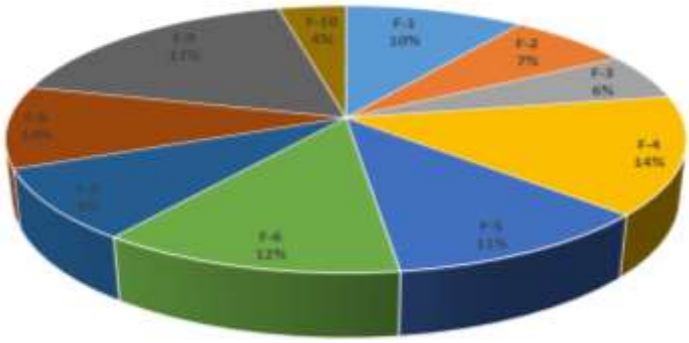

Figure 2: Distribution of bacteria isolated according to school canteens studied in this study

Antibiotics susceptibility testing of bacterial isolates.

A total of 161 bacterial isolates were obtained, and a subset of 42 isolates was selected for further investigation based on their distinctive morphology. The selection process involved evaluating the physical characteristics of the bacterial colonies, including size, shape, color, texture, and edge morphology. These features are commonly used to differentiate bacteria based on their phenotype. The antibiotic susceptibility patterns of bacterial isolates obtained from the study were evaluated using six different antibiotics, namely Ampicillin, Penicillin-G, Cotrimoxazole, Clindamycin, Erythromycin, and Cephalothin. The results showed that the bacterial isolates exhibited varying resistance levels and susceptibility to the antibiotics tested.

The present study investigated the antimicrobial susceptibility patterns of bacterial isolates obtained from ten school canteens. The results revealed a significant difference in the size of the inhibition zone among the isolates, with diameters ranging from 10 mm to 40 mm (Figure 3). These findings suggest variations in the susceptibility of the bacterial strains to the tested antibiotics. On the other hand, many isolates displayed resistance to the antibiotics, indicating the possibility of multi-antibiotic resistance. This was supported by the results presented, which indicated that the bacteria were resistant to more than one antibiotic.

Figure 4 illustrates the antibiotic susceptibility of the isolated bacteria according to the antibiotics used. Among the antibiotics used, Ampicillin showed the highest resistance level, with 62% of the bacterial isolates being resistant, while 38% were sensitive to the antibiotic. Penicillin-G also demonstrated a significant resistance level, with 45% of the bacterial isolates being resistant, while 55% were sensitive. In contrast, the lowest level of resistance was observed with Cephalothin, with only 12% of the bacterial isolates being resistant, indicating that 88% were sensitive to the antibiotic. Cotrimoxazole and Clindamycin showed moderate resistance levels, with 33% and 29% of the bacterial isolates being resistant to the antibiotics, respectively. The remaining 67% and 71% of the isolates were sensitive to Cotrimoxazole and Clindamycin, respectively. Erythromycin demonstrated the lowest resistance level, with only 19% of the bacterial isolates being resistant, while 81% were sensitive to the antibiotic.

Figure 5 explains the susceptibility patterns of bacterial isolates to the antibiotics used in the current study showing a clear difference in their sensitivity to the antibiotics. Eight isolates were found to be 100% sensitive to the antibiotics tested, while one bacterium was resistant to all the antibiotics used in the study. The remaining isolates exhibited varying levels of resistance and susceptibility to the antibiotics tested, as shown in Figure 5.

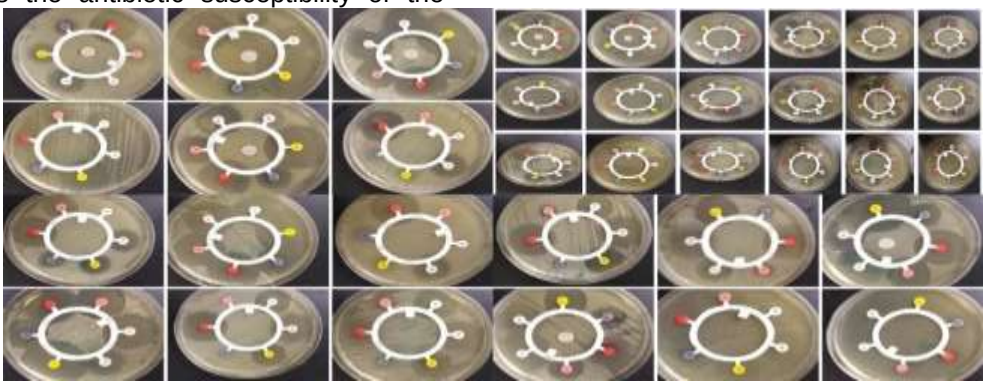

Figure 3: The inhibition zone(mm)of antibiotics test to bacterial isolated.

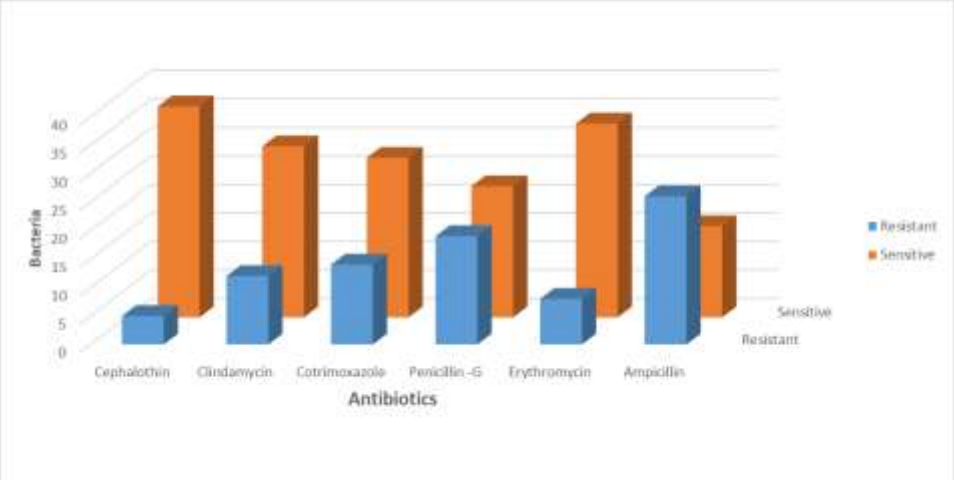

Figure 4: Antibiotic susceptibility of the isolated bacteria according to the antibiotics used.

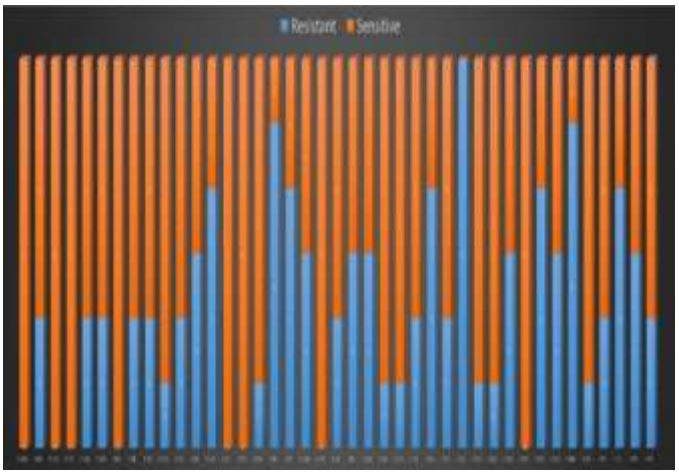

Figure 5: Antibiotic susceptibility according to the isolated bacteria

Disinfectant Susceptibility Testing of bacterial isolates

The study employed Al-Safe detergent, which is commonly used in schools, to assess its ability to inhibit bacterial growth, which was found to vary based on its concentration. The results showed that higher concentrations of the detergent led to larger inhibition zones on the surface of the agar used in the study. Figure 6. displays the inhibition zones at different concentrations of the detergent, with water used as the control. The study used five concentrations ranging from 1% to 100%, representing pure detergent without any added water. The results revealed that inhibition was non-existent at 1% concentration and increased as the concentration increased. The inhibition zone was 9.76 mm at 10% concentration and progressively increased to 13.82 mm, 19.6 mm, and 28.25 mm at concentrations of 25%, 50%, and 100%, respectively shown in (Figure 7).

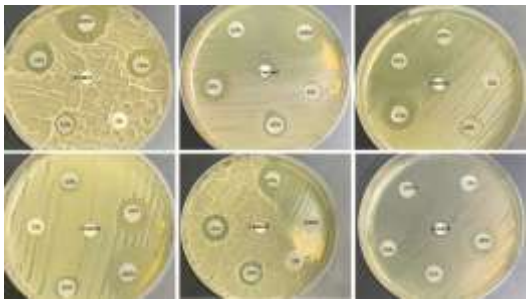

Figure 6: **Zone of inhibition of disinfectant susceptibility testing to bacterial isolates**

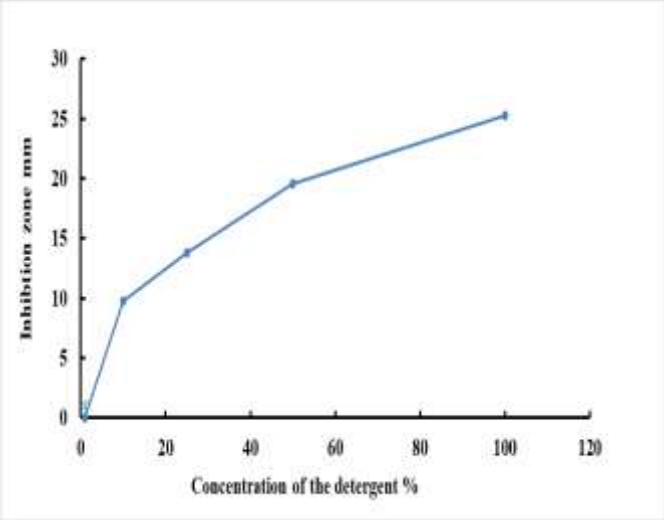

Figure 7: The average inhibition zone of disinfectant susceptibility (mm)

Hemolytic Activity of Bacteria on blood agar

The present study aimed to investigate hemolytic activity in bacteria isolated from the school canteen. The study results showed that out of the forty-two bacterial isolates, a significant proportion exhibited hemolytic activity.

Specifically, fourteen isolates (33.3%) (14/42) were found to have strong hemolytic activity, as evidenced by β hemolysis (Table 4.4) (Figure 4.10). Meanwhile, eleven isolates (26.9%) (11/42) showed moderate hemolytic activity, indicated by α hemolysis Table 4.4) (Figure 4.11). In contrast, no hemolytic activity was observed in seventeen of the isolates (40.47%) (17/42), as demonstrated by γ hemolysis (Table 4.4) (Figure 8).

Figure 8: Beta, Alpha, and Gamma hemolytic activity of isolated bacteria on blood agar. Phenotypic identification

Morphological Shape

The Gram-staining results showed that all isolates exhibited both Gram-positive and Gram-negative characteristics. These findings suggest the need for further characterization and identification of the bacterial isolates based on additional biochemical and molecular analyses. The results of the study showed that out of the 42 isolates, 32 were positive for the Gram stain, indicating that these bacteria have a thick peptidoglycan layer in their cell wall, whereas 10 isolates were negative for the Gram stain, indicating that these bacteria have a thinner layer or no peptidoglycan layer in their cell wall. The morphological and Gram-staining techniques employed in this study are critical tools for the initial screening and classification of bacterial isolates, providing important information for subsequent investigations of their ecological, medical, and industrial significance.

Molecular Identification of the Isolated Bacterial Strains

The extraction of high-quality genomic DNA is a crucial step in many molecular biology techniques, including PCR, sequencing, and genotyping. In this study, the genomic DNA was extracted from bacterial isolates, and the quality of the DNA was assessed by examining it under a UV transilluminator. The absence of smearing in the DNA sample is an indicator of good-quality DNA, as smearing can be an indication of DNA degradation or contamination. The yield of DNA obtained from the bacterial isolates was also determined. The high-quality genomic DNA obtained in this study is essential for downstream molecular biology applications, as it ensures accurate and reliable results. The successful isolation of good-quality genomic DNA depends on several factors, including the type of sample, the extraction method used, and the quality control measures taken.

In this study, PCR amplification of ribosomal DNA was performed using universal forward and reverse primers specific to 16S rDNA. The resulting PCR product was a fragment of approximately 500 bp, as shown in Figure 10, which is consistent with the expected size for this region in bacteria.A phylogenetic analysis was performed to investigate the variability within the amplified regions. The amplified PCR product was visualized by running it on a 1% agarose gel and observing it under a UV transilluminator, as shown in Figure 9.

In this study, the 16S rRNA gene sequences of all 42 bacteria were amplified and subjected to sequencing. The results revealed that the bacterial isolates belonged to different genera, with the majority being members of *Bacillus* sp (14 strains) and *Staphylococcus* sp (11 strains). Other genera identified included *Planomicrobium okeanokoites* (4 strains), *Pseudomonas* sp (2 strains), *Arthrobacter* sp (2 strains), *Stutzerimonas stutzeri, Escherichia fergusonii, Endophytic bacterium, Salinicoccus sp, Lysinibacillus sinduriensis, Enterobacteriaceae bacterium,* and *Bacterium CulaeenE9S*.

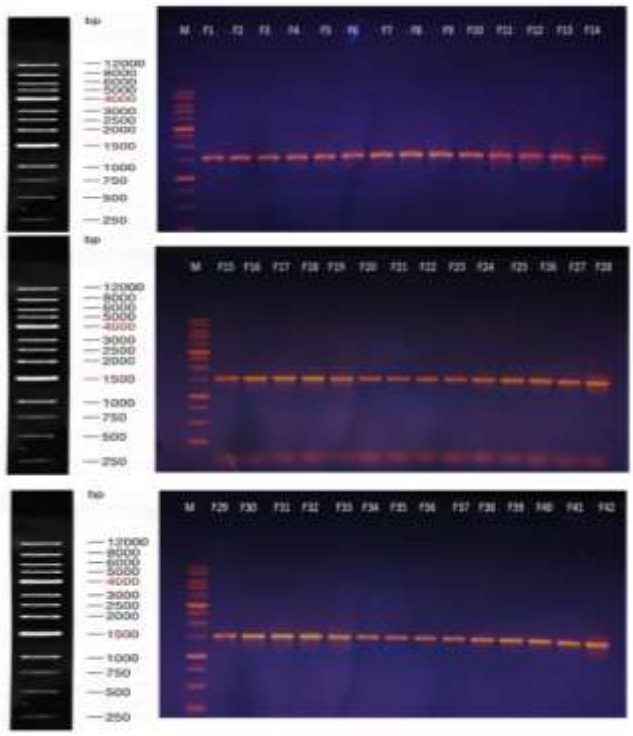

Figure 9: PCR product of 42 16s rRNA gene of different isolates from school canteen swaps samples,M; Genesta™ 1kb DNA Ladder, F1to F42; isolates.

DISCUSSION

At the start of every school year, various risks are associated with children's exposure to different foods at school or contaminated homemade lunches, leading to increased food poisoning cases and illnesses like colds and flu. Studies in different countries have reported outbreaks of foodborne diseases in schools, such as *E. coli* infection in Japan and Brazil, Salmonella poisoning in France, and norovirus in Germany. However, few studies have investigated food sources, personal hygiene, water, and practices related to food-borne diseases in Saudi Arabian schools. The Ministry of Health (MOH) and the local food sector in Saudi Arabia recognize the dangers of foodborne diseases and infections. They are working to identify the bacteria responsible for such illnesses. Previous national and international studies have investigated the presence of pathogenic microbes in school canteens. However, the traditional approach of identifying and detecting food-borne gastroenteritis outbreaks has limitations due to microorganisms' ability to modify their phenotypic features in response to environmental changes or genetic mutations.

The selection of different areas within the city of Jeddah and the inclusion of both government and private schools in the study can provide a more comprehensive representation of Primary Schools' Canteens in Jeddah. The large sample size of 100 surface swab samples collected from various locations within the school canteens with high contact frequency by children can increase the accuracy of the study's findings. The selection of a diverse set of schools located in different areas within the city of Jeddah, including both government and private institutions, can provide a representative sample of primary school canteens in the region. In previous studies, such diverse sampling approaches have provided a more comprehensive and accurate representation of the microbial diversity present in different environments (Lax et al. 2014; Bright *et al*. 2010). The inclusion of multiple schools and surfaces, which children frequently touch, can also improve the accuracy of identifying potential sources of pathogenic bacteria in school canteens (Rossi *et al*. 2018). The findings of this study can inform the development of appropriate control measures to prevent the spread of pathogenic bacteria in primary school canteens. Similar studies have shown the importance of implementing effective cleaning and disinfection protocols to prevent and control the spread of pathogenic microbes in school canteens (Egan et al. 2007; Mubarak 2019). Additionally, studies have highlighted the importance of regular monitoring and surveillance programs to ensure the effectiveness of these control measures (Nee and Sani 2011).

This study aimed to investigate the microbial contamination on various surfaces of school canteens, which are in direct contact with students' nutrition. The results of this study revealed the presence of a significant number of microorganisms on the canteen surfaces. These findings are consistent with previous studies that reported varying degrees of bacterial contamination in school canteens. For instance, a study conducted in Brazil found that school canteens had a higher bacterial contamination prevalence than other food service establishments (Almeida *et al*. 2014). Similarly, a study conducted in China reported a high incidence of pathogenic bacteria, including Staphylococcus aureus and Bacillus cereus, in school canteens(X 2011).

The results of this study revealed that the number of bacteria isolated from private schools 87(54.1) (n=161) is more than from government schools 74 (45.9) (n=161) (Figure 4.2). These findings highlight the importance of implementing effective cleaning and disinfection protocols in school canteens, particularly in private schools, to prevent the spread of pathogenic microbes. Similar studies have shown that implementing appropriate cleaning and disinfection protocols can significantly reduce the microbial load in school canteens (Egan *et al.,* 2007). Additionally, training staff on food safety and hygiene practices can also play a crucial role in reducing the spread of pathogenic bacteria in school canteens (da Vitória et al. 2018). However, the findings of this study contrast with those of a previous study, which found that the number of bacteria in public schools was higher than that in private schools (El-Kased and Gamaleldin 2020).

The present study investigated the antimicrobial susceptibility patterns of bacterial isolates obtained from ten school canteens. The results of the antibiotic susceptibility testing showed that the bacterial isolates exhibited varying levels of resistance and susceptibility to the antibiotics tested. The isolates showed a significant difference in the size of the inhibition zone, with diameters ranging from 10 mm to 40 mm, indicating variations in the susceptibility of the bacterial strains to the tested antibiotics. These findings are consistent with previous studies that have also reported variations in bacterial susceptibility to antibiotics (Admas et al. 2020; Edrees and Anbar 2021; Fardsanei et al. 2021). The findings of this study suggest that the bacterial isolates obtained from school canteens exhibited varying levels of susceptibility to the tested antibiotics, as demonstrated by the significant difference in the size of the inhibition zone. The diameter of the inhibition zone is an indicator of the effectiveness of the antibiotic in inhibiting the growth of the bacterial strain. A larger inhibition zone suggests a higher susceptibility to the antibiotic, while a smaller or absent inhibition zone indicates resistance. This variation in susceptibility could

be due to several factors, including the presence of genetic resistance mechanisms, differences in bacterial species, and variations in the concentration and potency of the antibiotics used.

Among the antibiotics tested, Ampicillin showed the highest resistance level, with 62% of the bacterial isolates being resistant, while 38% were sensitive to the antibiotic. This finding is in line with previous studies that have reported high levels of resistance to Ampicillin in bacterial isolates from various sources (Chehabi et al. 2019; Fiaz et al. 2021; Gessew et al. 2022; Al-Maaqar et al. 2022; Alotaibi et al. 2022). Penicillin-G also demonstrated a significant level of resistance, with 45% of the bacterial isolates being resistant, while 55% were sensitive. This finding is consistent with previous studies that reported varying levels of resistance to Penicillin-G in bacterial isolates (Ikken et al. 2020; Gandra et al. 2019). Cephalothin demonstrated the lowest level of resistance, with only 12% of the bacterial isolates being resistant, indicating that 88% were sensitive to the antibiotic. This finding is consistent with previous studies that reported high susceptibility levels to Cephalothin in bacterial isolates (Tartor et al. 2021). Cotrimoxazole and Clindamycin showed moderate resistance levels, with 33% and 29% of the bacterial isolates being resistant to the antibiotics, respectively. The remaining isolates were sensitive to these antibiotics. This finding is consistent with previous studies that have been reported (Abreu et al. 2022; Awuor et al. 2023; Al-Masaudi et al. 2020; Al-Masaudi and Al-Maaqar 2020).

The results also indicated that a considerable number of the bacterial isolates were resistant to the antibiotics tested, indicating the possibility of multi-antibiotic resistance. Multi-antibiotic resistance is a significant public health concern as it limits the treatment options for bacterial infections and increases the risk of treatment failure and the spread of infections. Multi-antibiotic resistance, also known as multidrug resistance (MDR), is a growing public health concern that limits the treatment options for bacterial infections. The World Health Organization (WHO) has identified MDR as one of the top ten global health threats and estimates that it could cause 10 million deaths annually by 2050 if not addressed (Medina *et al*. 2020). The emergence of MDR bacteria is a consequence of several factors, including overuse and misuse of antibiotics, poor infection control practices, and the spread of resistance genes through horizontal gene transfer (Ahmad et al. 2021; Jian et al. 2021).

School canteens and other communal settings can serve as potential reservoirs and sources of transmission of antimicrobial-resistant bacteria. Several studies have reported the presence of resistant bacteria in food, water, and environmental samples from school canteens (Ahmad et al. 2021; Alex 2019; Ologbosere and Ekhaise 2023). Therefore, continuous monitoring of antimicrobial resistance in school canteens and implementing appropriate measures, such as good hygiene practices, proper sanitation, and judicious use of antibiotics, are necessary to prevent and control the spread of resistant bacteria.

The present study evaluated the disinfectant susceptibility of bacterial isolates obtained from school canteens using Al-Safe detergent, commonly used in schools. The study aimed to assess the ability of the detergent to inhibit bacterial growth at different concentrations. The outcome displays the inhibition zones at different detergent concentrations, with water used as the control. The results revealed that inhibition was non-existent at 1% concentration and increased as the concentration increased. The inhibition zone was 9.76 mm at 10% concentration and progressively increased to 13.82 mm, 19.6 mm, and 28.25 mm at 25%, 50%, and 100%, respectively. The results showed that higher concentrations of the detergent led to larger inhibition zones on the agar surface used in the study. These findings are consistent with previous studies that have reported the effectiveness of detergents in inhibiting bacterial growth(Weese 2021; Gamaleldin 2020). The present study provides valuable insights into the disinfectant susceptibility of bacterial isolates using Al-Safe detergent. The results suggest that the concentration of the detergent is an important factor in determining its effectiveness in inhibiting bacterial growth.

The results of this study indicate that a significant proportion of the bacterial isolates obtained from school canteen surfers exhibit hemolytic activity. Specifically, 33.3% of the isolates showed strong hemolytic activity, while 26.9% showed moderate hemolytic activity. Hemolytic bacteria are a group of bacteria that can cause the breakdown of red blood cells, a process known as hemolysis. Hemolytic bacteria produce hemolysins, which are toxins that can damage the cell membranes of red blood cells, leading to their lysis. Hemolysins are classified into three types based on their activity on red blood cells: alpha, beta, and gamma hemolysins. Alpha hemolysins cause partial hemolysis, resulting in a greenish discoloration of the blood agar around the bacterial colonies. Beta hemolysins cause complete hemolysis, creating a clear zone around the colonies. Gamma hemolysins do not cause hemolysis and do not produce a zone of clearing around the colonies. These findings are consistent with previous studies that have reported the presence of hemolytic bacteria (Kalathingal et al. 2009; Braito et al. 2004; Tokgöz et al. 2005).

Regarding identification, primary school canteens can be potential sources of foodborne illnesses if proper food safety practices are not implemented. The presence of pathogenic bacteria in these environments can pose a risk to the health of students and staff who consume food from these canteens. Therefore,

identifying and characterizing the bacterial strains present in these environments is essential for ensuring food safety. Microbial and molecular identification techniques in this study will provide valuable information on the bacterial strains in primary school canteens. Microbial identification techniques involve isolating and characterizing bacterial strains based on their morphological and biochemical properties. On the other hand, molecular identification techniques involve using molecular biology tools, such as PCR amplification and sequencing, to identify bacterial strains based on their genetic characteristics.

The morphological and Gram-staining analyses are essential techniques used in microbiology for bacterial identification and classification. Morphological analysis involves the observation of bacterial colonies on various types of media, which can provide information on the physical characteristics of the cells, such as size, shape, color, texture, and growth pattern. The ability of bacteria to grow on specific types of media depends on their nutritional requirements and metabolic characteristics. For instance, blood agar is a differential medium used to distinguish between different types of bacteria based on their hemolytic properties. Nutrient agar is a general-purpose medium that supports the growth of many types of bacteria, while MacConkey agar is a selective and differential medium used to isolate and identify gram-negative bacteria based on their lactose fermentation ability (Mac Faddin 1985). Gram staining is another commonly used technique for bacterial identification. These techniques are crucial for identifying and classifyingof bacterial isolates in various fields, including medicine, environmental science, and the food industry. Several national and international studies have used gram staining as a tool to identify and classify bacteria in school environments, particularly in school canteens where food-borne illnesses are a significant concern. The results of these studies have provided valuable insights into the prevalence of pathogenic bacteria in school settings and have contributed to the development of effective strategies for preventing and controlling outbreaks of food-borne diseases. In the context of the statement, the use of gram staining in previous studies is being acknowledged and the statement emphasizes that the results obtained from the current study are consistent with the findings of those previous studies. This demonstrates that gram staining is a reliable method for identifying and classifying bacteria in school environments, and the current study's results support the existing body of knowledge on the prevalence of pathogenic bacteria in schools(Tshikhudo et al. 2013; Alharbi et al. 2019; Fareid et al. 2011; Davis and Mauer 2010; Brągoszewska et al. 2018; Park et al. 2021).

Molecular identification of the bacterial isolates was performed using PCR amplification of ribosomal DNA, specifically the 16S rRNA gene. The sequencing results revealed that the bacterial isolates belonged to different genera, with the majority being members of Bacillus sp and Staphylococcus sp and another bacterium. These findings are consistent with previous studies that have reported Bacillus sp in schools or other environments (Forero et al. 2018; Reyes et al. 2007), Staphylococcus sp. (Rijal et al. 2008), *Escherichia fergusonii*(Tang et al. 2023), *Planomicrobium okeanokoites* (Saini et al. 2023), Pseudomonas sp(Su et al. 2017), Arthrobacter sp(Ren et al. 2015), and Entero bacteriaceae bacterium(Sanneh et al. 2018).

Overall, this study is important for identifying and characterizing the bacterial strains present in primary school canteens in the Jeddah Region of Saudi Arabia. The results of this study can be used to develop appropriate food safety measures to prevent the spread of food borne illnesses in these environments and ensure the health and safety of students and staff.

CONCLUSIONS

This study provides evidence that various types of bacteria contaminate all the surfaces analyzed in ten schools. The results indicate that children attending both private and public schools are at a high risk of contracting serious bacterial infections due to the abundance of microorganisms present throughout the school environment. Private schools appear to have a higher concentration of pathogenic bacteria than government schools. As a result, ministries of education, environmental affairs, and health worldwide should prioritize improving the school environment for these children. School authorities should be educated on the benefits of using disinfectants to eliminate widespread microorganisms, particularly on surfaces that young school children come into contact with. Children should also be advised to frequently wash their hands and sanitize their belongings. Schools should supply each class with hand sanitizer and ensure that there is enough soap in the toilets. They should also strictly monitor the cleaning of the toilets and classrooms. Continuous education and reminders should be provided to children to encourage them to maintain cleanliness in their surroundings. By utilizing the findings of this research, we can develop new strategies to enhance the hygiene of our schools and create a healthier and safer learning environment.

Supplementary materials

Not applicable

Author contributions

Fahad Dhaifallah Al-Shareef was involved in all the experiments, contributed to drafting the manuscript, and analyzed the data. Meanwhile, Mohammad Hussein Fakieh, Mohamed Morsi Mohamed Ahmed conceived the idea, planned, designed, and coordinated the

experiments.

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Conflict of interest
The present study was conducted without any conflicts of interest, according to the authors.

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