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Comparison of HlgB, hysA, MecA, and papAH and Other Genes between E. Coli and S. Aureus Bacteria Isolated from Pediatric with Diarrhea and from House Flies

Conflict of interest: nothing to declare.
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Abstract

Introduction. The majority of infections in children with diarrhea are caused by gram-negative bacteria; include Pseudomonas aeruginosa, Klebsiella pneumonia,and Escherichia coli.
Purpose. To prove that house flies transmit bacteria that cause diarrhea in children by comparing the genes between bacteria isolated from house flies and bacterial samples from children with diarrhea.
Materials and methods. The research period is from July 2022 to January 2023 in Thi-Qar Governorate. After morphological and microscopic diagnosis and biochemical tests, the following results were obtained.
Results. The current study showed that the most isolated bacteria is E. coli with a percentage of (24.3%), followed by S. aureus (17.0%), followed by Klebsiella spp. (13.15%), while the least isolated bacteria are Micrococcus (3.94%). When comparing the bacteria present in flies with the bacteria isolated from pediatric patients with diarrhea, these bacteria, the current study showed that the predominant bacteria was Escherichia coli from diarrhea with a percentage of (44.63%), and Escherichia coli was (36.27%) in the dominant flies. Staphylococcus aureus in flies followed (25.49%), while diarrhea followed (16.53%). On the other hand, the least dominant bacterial species was S. pyogen in both diarrhea and diarrheal flies with (8.82%) and (9.09%), respectively. As for the genetic diagnosis, it was as follows S. aureus bacteria were the results of genes A (hlgB- lip – hysA) appeared in all samples negative (Five samples from sick children infected with diarrhea and five from houseflies). The following genes, (16SrRNA gene, mecA gene) all ten samples showed positive results. As for E. coli bacteria, results appeared as follows (16SrRNA -papAH - sfaS-rfc- iha- mcr1) all ten samples showed positive results.

Conclusions. This study proved House flies are carriers of pathogenic bacteria that cause diarrhea in children under the age of ten through genetic diagnosis of the bacteria, S. auers, E. coli (through the 16SrRNA gene) and some other genes.
Keywords: gene, pediatric, houseflies, diarrhea, Musca domestica

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Сравнительный анализ наличия HlgB, hysA, MecA, papAH и других генов в изолятах бактерий E. coli и S. aureus у детей с диареей и домашних мух

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Резюме

Введение. Большинство инфекций, сопровождающихся диареей, у детей вызываются грамотрицательными бактериями, включая Pseudomonas aeruginosa, Klebsiella pneumonia и Escherichia coli.
Цель. Доказать, что домашние мухи являются переносчиками бактерий, вызывающих диарею у детей, путем сравнения генов бактерий из изолятов домашних мух и из образцов, полученных от детей с диареей.
Материалы и методы. Период проведения исследования – с июля 2022 г. по январь 2023 г. в мухафазе Ти-Кар. Выполнены морфологическая и микроскопическая диагностика, а также биохимические исследования.
Результаты. Настоящее исследование показало, что наиболее часто выделяемой бактерией является E. coli (24,3%), за ней следует S. aureus (17,0%) и Klebsiella spp. (13,15%); реже всего выделяли Micrococcus (3,94%). В ходе данного исследования при сравнении количества бактерий, обнаруженных в мухах, с количеством бактерий в изолятах педиатрических пациентов с диареей было установлено, что в каловых массах детей с диареей преобладали Escherichia coli в 44,63% случаев, а в мухах – Escherichia coli (36,27%). Содержание Staphylococcus aureus в мухах составило 25,49%, а в каловых массах – 16,53%. Наименее распространенными как в каловых массах детей с диареей, так и в мухах были S. pyogen – 8,82% и 9,09% соответственно.

При анализе образцов, содержащих S. aureus, на наличие генов A (hlgB- lip – hysA) (пять изолятов от детей с диареей и пять изолятов из мух) во всех случаях был получен отрицательный результат. При анализе этих же образцов на наличие генов 16SrRNA gene, mecA gene положительный результат был получен во всех десяти случаях. Анализ образцов с E. coli на наличие генов 16SrRNA -papAH - sfaS- rfc- iha- mcr1 дал положительный результат для всех десяти исследованных образцов.

Выводы. На основании результатов генетической диагностики бактерий S. aureus и E. coli по гену 16SrRNA и некоторым другим генам подтверждено, что домашние мухи являются переносчиками патогенных бактерий, вызывающих диарею у детей в возрасте до десяти лет.

Ключевые слова: ген, педиатрические пациенты, домашние мухи, диарея, Musca domestica

■ INTRODUCTION

House flies (Musca domestica Linnaeus) are one of the most common species in the world, Musca domestica followed by a family of Muscidae from order diptera, accounting for almost 90% of the flies found in human and animal living places. This is due to the expansion of the production of poultry fields and lives of cattles, because these environments provide breedin grounds for the multiplication of many of the insect pests as well as the lack of sewage networks in some residential areas and the random disposal of waste and other factors that affect the proliferation and reproduction [1]. House flies carry infections from unclean areas, including trash and sewage, to human and animal food by their excrement, vomit, or other bodily parts. This allows them to spread disease [2]. The fly has a very sensitive mouth, and when it touches a human's skin, it swallows the secretions instead of biting the victim. Sweat, proteins, carbohydrates, salts, sugars, and other substances, as well as fragments of dead skin that continue to peel off the surface of human skin, pique the attention of the flies [3].

Global estimates show that among all diarrheal causes, the prevalence of particular forms of bacterial diarrhea is 10% to 25% for E. Coli, 10% for Shigella, 3% for Salmonella, and 3 to 6% for Campylobacter [4] and it was calculated that 31% of diarrhea cases in the United States were caused by bacteria. The majority of infections in children with diarrhea are caused by gram-negative bacteria; similar rates, frequency, and pathogens include Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, and the gram-positive organism. Mortality can be independently predicted by Staphylococcus aureus. Worldwide, Staphylococcus aureus is the primary cause of gram-positive infections related to diarrhea in children and a frequent cause of septicemia resistant to methicillin. In some burn centers, Staphylococcus aureus (MRSA) has become the predominant pathogen, while vancomycin-resistant enterococci (VRE) are less common but seem to have a higher pathogenicity [5].

■ PURPOSE OF THE STUDY

To prove that house flies transmit bacteria that cause diarrhea in children by comparing the genes between bacteria isolated from house flies and bacterial samples from children with diarrhea.

MATERIALS AND METHODS

Samples Collection

Flies were collected from (segmentation, chicken and fish shops, markets hospitals and house) and for the cities of Al-Shatrah and Al-Nasiriyah in Thi-Qar Governorate during the study period from July 2022 to January 2023; Where 350 flies were collected and 126 flies showed the presence of bacteria on their outer and inner surface. The flies are caught directly through the collection bottles, and after transferring them to the laboratory, we take the following steps, which is placing the flies in the refrigerator for 3 minutes at a temperature of 0 degrees Celsius to inactivate them, and then we isolate the bacteria from the flies, as shown below. As for the percentage of children with diarrhea, a total of two hundred stool samples were also collected from children with diarrhea ranging in age from days to ten years, and of both sexes.

Isolation of Pathogenic Bacteria from Flies

The flies' samples were placed in the refrigerator at the degree of temperature for 15 minutes for the purpose to inactivate their activity; in order to isolate bacteria from them (flies) and they were washed by placing them in 1 ml of sterile water for one minute twice, then were placed in normal saline with a concentration 0.85% (2 ml) and then transferred 0.1ml to the plates containing the appropriate culture for a whole day, the plates were cultured for microorganisms at 37 °C. While isolating bacteria from the inner surface, the outer surface of fly samples is initially sterilized by washing them with ethyl alcohol at a concentration of 70%, then gently washed with sterile water using a dissecting microscope, and the insect was dissected using sterile dissection tools to extract the intestines and then crushed. ml of saline solution for the purpose of soaking, then take 0.1 ml of the infusion and plant on the head [6].

Isolate of Pathogenic Bacteria from House Fly

After collecting the bacteria from the outer surface and the inner surface of the flies, they were grown on two mediums. As well as in the diarrhea samples for children under the age of 10 years from Shatra General Hospital and cultured on special culture media (blood agar; MacConkey agar) Where the bacteria were present was diagnosed. As well as biochemical and bacterial growing tests by Gram stain and they were finally diagnosed by API-20 and VITEC Finally, the genetic diagnosis [7].

Diagnosis with the Following Steps

Colony properties was tested such as shape of the colonies, size, color, borders and texture of colonies.

F. The Diagnosis is the following steps:

- Microscopic examination.
- Biochemical test.
- API-system (analytical profile index for E. coli and S. aureus).
- VITIC 2 compact.
- Antibiotic susceptibility.
- Molecular diagnosis.

RESULTS

Identification of Isolated Bacteria from Flies

The present study showed the high isolated bacteria was E. coli 24.3%, followed S. aureus 17.0%, followed Klebsiella spp. 13.15%, while the lowest isolated bacteria was Micrococcus 3.94%. as show in Fig. 1.

Identification of Isolated Bacteria from Pediatric Stool

The present study showed the high isolated bacteria was E. coli 30.85%, followed Klebsiella spp. 14.28%, followed S. aureus 11.42%, while the lowest isolated bacteria was Y. enterocolitica 1.14%. as show in Fig. 2.

A Comparison between Dominant Bacteria Isolated from Flies and Stool

The present study showed the high dominant bacteria were E. coli from diarrhea 44.63%, also in flies the high dominant was E. coli 36.27%. followed S. aureus in flies 25.49%, while in diarrhea 16.53%. in the other hand the lowest dominant bacteria were

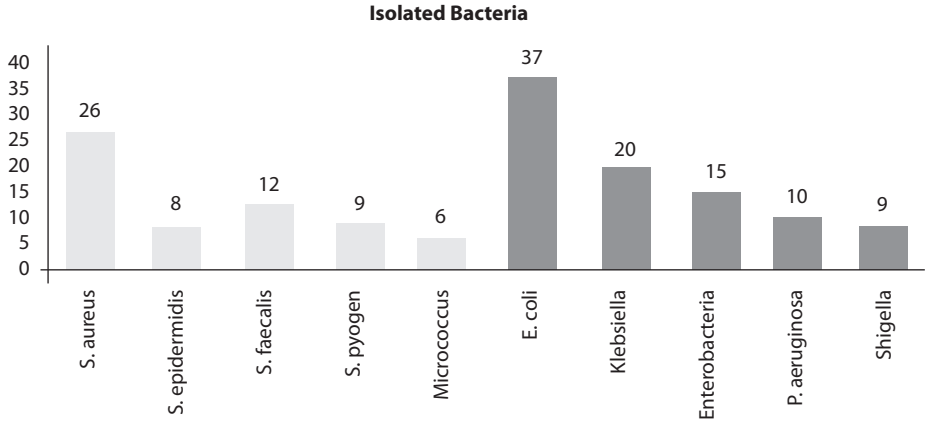


Fig. 1. Identification of isolated bacteria from flies

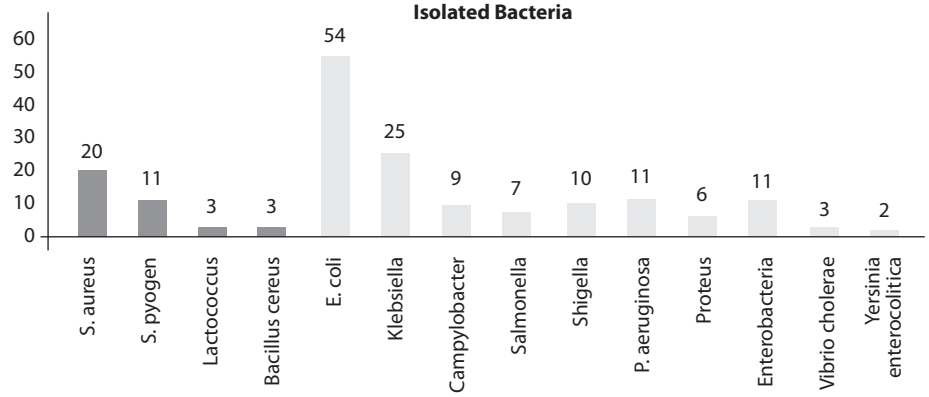


Fig. 2. Identification of Isolated Bacteria from Pediatric Stool

A comparison between dominant bacteria isolated from flies and stool

Source Bacteria	Flies		Diarrhea		Total	
	No.	%	No.	%	No.	%
S. aureus	26	25.49	20	16.53	46	20.62
S. pyogen	9	8.82	11	9.09	20	8.97
E. coli	37	36.27	54	44.63	91	40.81
Klebsiella	20	19.61	25	20.66	45	20.18
P. aeruginosa	10	9.80	11	9.09	21	9.42
Total	102	45.74	121	54.26	223	100
$CalX^2 = 2.596$	$TabX^2 = 9.49$		DF=4		p. value 0.628	

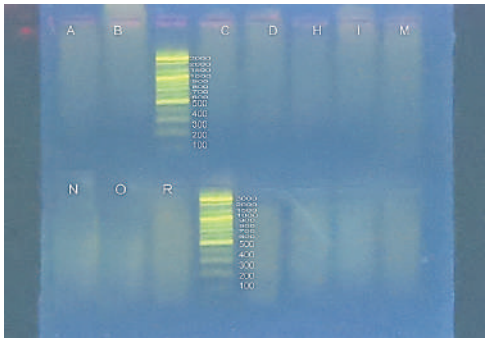
S. pyogen in both flies and diarrhea 8.82%, and 9.09% respectively. Additionally, as shown in table, the study found no significant difference between flies and stool at p. value <0.05 (Table).

■ DISCUSSION

Comparison of Bacteria Isolated from Flies and Diarrheal Patients

When you see Figure 1 and 2, we find the present study showed the high dominant bacteria were E. coli from diarrhea 44.63%, also in flies the high dominant was E. coli 36.27%. followed S. aureus in flies 25.49%, while in diarrhea 16.53% in the other hand

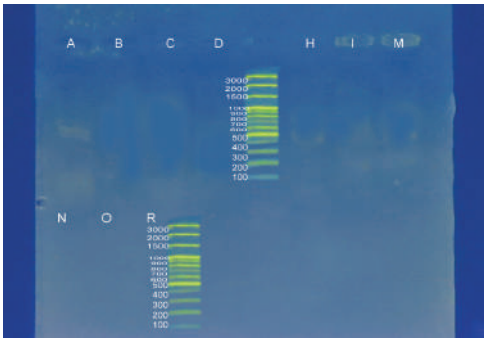
Gene Results
Genes S. Aureus
HlgB Gene
PCR Product size: 792 bp. All samples appeared negative percentages (100%)



A-B-M-C-D-H-I-M
N-O-R-M
Gene: HlgB Gene, PCR Product size: 792 bp
TBE Buffer (1x), Agarose concentration (1.5%), Orange Dimond Dye mt Promega
Time: 40 min (70-volt, 85 mA)

Fig. 3. Agarose gel electrophoresis of HlgB Gene amplification

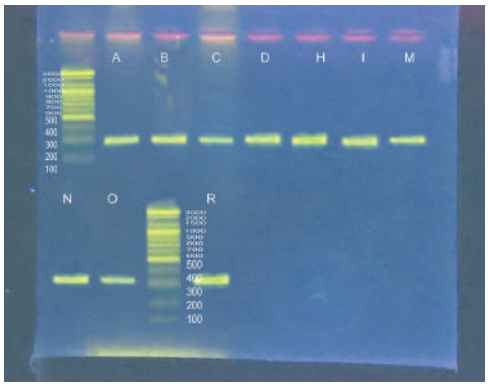
Other Genes
The results of other genes (hlgB- lip – hysA) appeared in all samples negative by (100%). Five samples from sick children infected with diarrhea and five from houseflies as show Fig. 4.



A-B-C-D-M-H-I-M
N-O-R-M
Gene: hysA Gene, PCR Product size: 936 bp
TBE Buffer (1x), Agarose concentration (1.5%), Orange Dimond Dye mt Promega
Time: 40 min (70-volt, 85 mA)

Fig. 4. Agarose gel electrophoresis of hysA Gene amplification

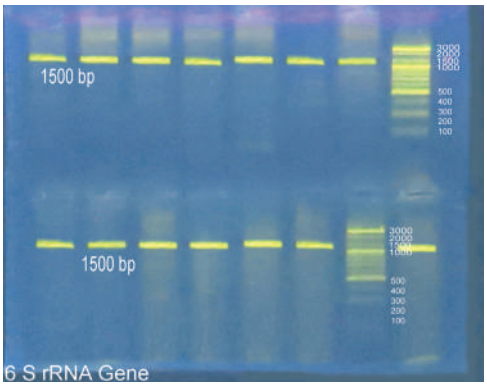
MecA Gene
PCR Product size: 310 bp. All samples appeared negative percentages (100%)



M-A-B -C-D-H-I-M
N-O-M-R
Gene: MecA Gene, PCR Product size: 310 bp
TBE Buffer (1x), Agarose concentration (1.5%), Orange Dimond Dye mt Promega

Fig. 5. Agarose gel electrophoresis of MecA Gen amplification

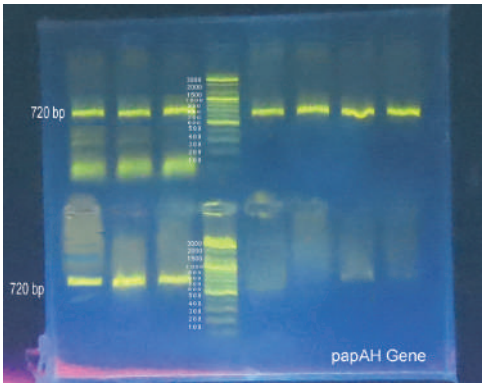
Genes E. Coli
Gene 16 S rRNA
PCR Product size: 1500 bp. all ten samples were positive percentages (100%)



11-12-13-14-15-16-17-M
18-19-20-A-B-C-M-D
Gene 16 S rRNA, PCR Product size: 1500 bp
TBE Buffer (1x) agarose concentration (1.5%), Orange Dimond Dye mt Promega
Time: 14 min (70-volt, 85 mA)

Fig. 6. Agarose gel electrophoresis of 16 S rRNA amplification

Other Genes
The results of other genes (papAH - sfaS- rfc- iha- mcr1) showed a positive result for all samples by (100%).



11-12-13-M-14-15-16-17
18-19-20-M
Gene: papAH, PCR Product size: 720 bp
TBE Buffer (1x), Agarose concentration (1.5%), Orange Dimond Dye mt Promega

Fig. 7. Agarose gel electrophoresis of papAH amplification

the lowest dominant bacteria were S. pyogen in both flies and diarrhea 8.82%, and 9.09% respectively. The study also, as show in table.

In a study carried out in Al-Nasiriyah city by Lhwak and Abbas [8], which also converged with our findings, Al-Hilali [9] isolated the following species. These results were in agreement with K. pneumoniae, S. aureus, P. aeruginosa, P. mirabilis, Enterococcus spp., S. saprophyticus, and Citrobacter spp. [10]. This result agreement with quondam studies in by Blazar et al. [11] mentioned Pediatric Patients with Isolated Bacterial Species Escherichia. Coli (36.58%) and Salmonella species (2.44%) as well as Pseudomonas species (2.44%) which were the least common, others are Staphylococcus species (26.83%), Shigella species (14.64%), Streptococcus species (17.07%), while, Kababian et al. [12], this research was conducted in Qom Province, Central Iran. E. coli; Pseudomonas aeruginosa; Klebsiella pneumonia; Proteus mirabilis and Staphylococci aureus have been documented from the house flies' exterior surfaces.

This result disagreement with study by Pava-Ripoll et al. [13], another study by Jabber et al. [14], the study's findings were somewhat similar to those reported by Singhal et al. [15], who stated that among Of the 292 bacteria, it was the lowest percentage, E. coli, K. pneumoniae and Citrobacter diversus; also disagreement with our findings depended on location was due to many reasons including the differences of sewage systems in houses, hospitals and vegetable market sites, in addition to the increase of waste in each of the above sites, which all lead to the spread of houseflies carrying these bacteria as well as the spread of bacteria itself .the reason may be due to the appropriate temperatures for the growth and presence of bacteria, in addition to the abundance of flies during certain months of a year, Numerous causal routes, including direct impacts on bacterial proliferation and indirect effects, exist for how temperature affects the spread of bacteria. The frequency of bacteria-carrying insects rises throughout the warm months as their activity does.

Genetic Comparison

Genes Isolated from S. Aureus

We found that each of the following genes (hlgB- lip – hysA) appeared in all samples negative (Five samples from sick children infected with diarrhea and five from houseflies). These results agreed with all of the results McAdow et al. [16], Abbas et al. [17]. The reason for the similarity in these results is due to several reasons, including that this gene is present in bacteria in a small percentage, as well as the isolated environment, including bacteria.

This finding is not consistent with both Brody et al. [18], Grunenwald et al. [19]. The difference is mainly due to the place of isolation and the environment of the bacteria from which it was isolated. The action of these genes is more effective in skin infections, as they are found more in bacteria isolated from those areas, but the percentage of their effectiveness depends on the isolation environment, the place of isolation, and the environmental conditions.

All ten samples showed positive results 16SrRNA gene. Either the results of the gene appeared mecA gene, all ten samples showed positive results in similar Comparing the results of this study to those of a study done in Iran, Fatholahzadeh et al. [20], found that the percentage of MRSA infection outbreaks was 67.2%. However, MRSA prevalence varies greatly throughout nations, which may be a result of disparate infection management

strategies [21]. Similarly, MRSA is the most prevalent bacteria in traumatology, burn, and resuscitation departments [22, 23]. The incidence of MRSA among S. aureus was found to be 83.7%, 84%, 88%, and 94.3%, respectively, in other local studies conducted in the same field. These studies included, Abd-Elateef [24], Babakir-Mina et al. [25], and Al-Dahbi and Al-Mathkhury [26].

However, in other investigations, such as Taha et al. [27] in Erbil, Abdullah [28] in Baghdad, and Rabelo et al. [29] in Brazil, low percentages of MRSA were found in S. aureus isolates. This research revealed that MRSA incidences were 30.24%, 41.54%, 42.3%, and 10%, respectively. In contrast, study by Santos et al [30] found that MRSA incidence in Brazilian hospitals ranged from 40 to 80%.

Genes Isolated from E. Coli

For many decades, butchers and slaughterhouses in Iraq have not grown significantly due to unfavorable economic policy. There are many butcher shops selling meat gained from animals living on farms inside Iraq. The slaughterhouse and the butcher shop are important places that serve the townspeople to buy various live livestock products. It would be logical to consider and include these sites as critical sites in infection control design for bacterial infection [31].

The results of all ten genes (five from pediatric patients with diarrhea and five from houseflies) appeared, all of which were positive. (Adhesins (sfaS), Capsule synthesis (rfc), These results agreed with both studies Bach et al. [32], and Bonardi et al. [33], and the collection place of butchers' shops and vegetable markets are all of the reasons for this similarity, in addition to the fact that this gene is activated in bacteria that live, especially in sewage water, which are close to those areas, and the method of slaughter that is close to sewage water and is a reason for the transfer of flies to those bacteria carrying those genes.

The results of these genes were, but at a lower rate, as in the study of Fukushima et al. [34], and Auda [35]. The reasons are due to the collection of these samples from cows only before the slaughter process in their place of living (barns), as the environment has an important role in the presence of bacteria that carry these genes over others when they multiply. And the proximity of pollution sources in which these bacteria exist.

This gene is responsible for cases of diarrhea, especially in children. For the following genes, which are responsible for virulence (papAH – iha). Where the results of all ten samples were positive, the study showed similarity with the study of both Dobrindt et al. [36]. Where all studies showed a high percentage of the presence of this gene and are associated with diarrhea samples, as it was found that these genes are activated in cases of diarrhea, i.e. the environment, the living conditions and the place of isolation cause the similarity. Studies showed less than 100% in some studies, as in Bonardi et al. [33] and A'iaz [31]. The method of isolation from a place other than diarrhea samples was a major reason for the absence of these genes, as well as the difference between countries in terms of different environmental conditions between countries.

In conclusion, we find that the genes of S. aureus bacteria were all negative in samples taken from flies and children with diarrhea, with the exception of the mec gene, which was all positive. As for the E. coli bacteria isolated from flies and children with diarrhea, it showed the presence of all the studied genes, and this confirms that house flies are one of the main causes of transmitting bacteria that cause diarrhea in children**.**

■ CONCLUSIONS

The study confirmed that bacterial isolates contaminated with houseflies and diarrhea samples recorded the highest percentage of Escherichia coli and Staphylococcus aureus, and the current study demonstrated that there is a positive relationship between contamination of flies and diarrhea samples.

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