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Detection of Verotoxic *Escherichia coli* isolated from the surface of fingerprint devices using conventional and rapid PCR techniques.

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ABSTRACT

Background: Time attendance fingerprint devices became used increasingly in many of government institutions such as universities , hospitals and airports. In such crowded places the chance of transmission the transitional diseases are fairly large. Verocytotoxigenic *E. coli* is one of pathogenic bacteria that can transfer by direct or indirect contact with few numbers and succeeded in causing infection. **Objective:** This paper aim to study and evaluation the possibility of transmission of bacterial infection via fingerprinting and detection of Verotoxic *Escherichia coli* by using conventional and rapid PCR techniques. **Results** : Twenty-four cotton swab collected from the surface of 12 fingerprint devices used in three hospitals and two universities in Baghdad city. Conventional and Rapid PCR techniques were used for detection of Verotoxic *Escherichia coli.* Results showed that 66.6 % (4) of swabs which collected from 6 devices from 3 hospitals and tested for VTEC by using rapid PCR technique were positive. While only 33.3% (2) of swabs tested by conventional PCR technique showed positive detection for VTEC. All the samples taken from the surfaces of devices used in universities were free of bacteria and the same results were obtained from using the conventional and rapid PCR techniques. **Conclusions**: The fingerprint devices which currently used in different places is associated with a risk of infection transmission of Verocytotoxigenic *E. coli* and the rapid PCR technique is preferred for the detection of these bacteria because it easy , do not need additional steps or kits for DNA extraction with low cost and short time compared to conventional method.

KEYWORDS: Fingerprint device; Infection transmission; *E. coli*; PCR technique

INTRODUCTION

Diarrheal disease which caused by *Escherichia coli* remains a major public health problem and because of the costly diagnostic procedures, the epidemiology of *E. coli* infections remains obscure in many parts of the world[1,2].There are five categories of diarrheagenic *E. coli* strains which recognized on the basis of clinical and epidemiological features [3].

Verotoxic *Escherichia coli* (VTEC) produce verocytotoxins (VT-1 and VT-2) which effect on Vero cells within host and they also called Shiga toxins (Stx) because they similar to the toxins produced by *Shigella dysenteriae* [4,5]. The infection with VTEC occurs via faecal- oral rout caused with mild to severe bloody diarrhea. Most studies report that medical and nonmedical devices such as thermometers , keyboard covers , pens ,mobile and other devices act as reservoirs of these pathogens [6,7], where hands be the main rout of transfer [8,9].

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Time attendance fingerprint device, which used for the purpose of organizing and prove the presence of the departure of the staff at work places, became used commonly in many government departments , such as airports, hospitals and universities. Process of capturing and reading of fingerprint requires physical contact between the finger or hand skin and the surface of the devices [9].

Environmental samples may contain very low numbers of VTEC, Unlike clinical specimens which contain high numbers of the pathogen present and without an enrichment step in the protocol of the isolation , these bacteria may not be identify , resulting in incorrect results [10,11]. Many of methods based on bacterial DNA have been reported for the detection and characterization of VTEC. The most commonly methods are includes the use of the conventional polymerase chain reaction (PCR) to amplify and detect a specific virulence factor in VTEC as sequences of vt genes (vtx1, vtx2) , a process that can take a number of hours [12] .

This current research studied the risk of transfer of VTEC (in doses sufficient to cause infection) through the fingerprinting devices and identification of these microorganisms by using two methods of PCR techniques.

MATERIALS AND METHODS

Swabs collection:

12 fingerprint device from 3 hospitals and 2 universities in Baghdad city were chosen randomly in this study. Two swabs were used for each surface of each device. All swabs were firstly moistened with Triton X-100 (0.1%) before passed over the surface of device. One swab inoculated in 10 ml tryptone soy broth (TSB) at 37°C for 24 h while another swab was kept frozen. Therefore, the total swab was 24 (12 devices × 2 swabs).

All swabs were processed immediately after return to the laboratory.

Bacterial DNA detection by PCR technique:

Tow procedure of PCR technique were used to detection of bacterial DNA: Conventional PCR and Rapid PCR.

A-Conventional PCR:

Bacterial DNA extraction:

Bacterial DNA was extracted from the swabs by using the PowerSoil DNA Isolation kit[13]. The cotton tips of frozen swabs were broken off directly into bead tubes and 60 µL of solution C1 had been added.The tubes were incubated at 65°C for 10 min and then shaken horizontally at maximum speed for 2 min. Centrifuged at 10,000 x *g* for 30 seconds at room temperature. The supernatant transferred to a clean collection tubeand 250 µL of solution C2was added and mixed for 5 seconds by vortex. Incubated at 4°C for 5 minutes. The remaining steps were performed as describe by the manufacturer. The obtained DNA stored frozen at 80°C until the PCR time.

Primers Design:

PCR was carried out for detection the virulent genes of VTEC by targeting specific Vt2 genes [14]. Required sizes and base sequences of amplified products for the specific primers (oligonucleotide sequence) are showed in Table 1.

Table 1: Primer designation.

Target gene	Primer designation and sequence (5' - 3')	Amplicon size (bp)
Vt2 (Forward)	CTTCGGTATCCTATTCCCGG	478
Vt2 (Reverse)	GGATGCATCTCTGGTCATTG	

PCR Amplification of Extracted DNA:

Amplification of bacterial DNA for Vt2 genes was carried out in a thermal cycler (Master cycler gradient, Germany) 10 µl of prepared bacterial DNA samples was added to 90 ml of PCR mixture (10 mMTris-HCl [pH 8.3], 50 mMKCl, 1.5 mM MgCl2, 0.25 mM of each deoxynucleoside triphosphate (dATP,dTTP, dGTP, dCTP) [Pharmacia Biotech Inc.], 20 PMol of primers and 1.5 U of *Taq*polymerase [Boehringer Mannheim Biochemicals, Indianapolis, Ind.])

The thermal cycling conditions for PCR process were 2 min for initial denaturation of DNA at 94°C followed by 30 cycles of 94°C for 1 min (denaturation) , 1 min for primer annealing at 55°C and 1 min (DNA synthesis) at72°C.

The amplified products of DNA were detected by agarose gel electrophoresis using 10 µl of PCR product on 1.5% agarose gel in TBE buffer for 45 min at 120V after using molecular size markers (DNA ladder marker,

Genei, Bangalore) in each gel. Then gel stained with 0.5 µg/ml ethidium bromide. Segments of amplified DNA were determined by using UV fluorescence.

B- Rapid PCR:

One ml of culture was taken in microfuge tube and heated for 10 min in boiling water. The microfuge tube, transferred immediately to ice was used for PCR. Amplification of bacterial DNA for Vt2 genes was carried out in a thermal cycler (Master cycler gradient, Germany) in the same way as described in the above.

RESULTS AND DISCUSSION

By using 2 different PCR techniques (conventional and rapid PCR) the results of this study showed that all swabs (12 swabs) collected from the 2 universities were free from Verotoxic *Escherichia coli*.66.6 % (4) of swabs which collected from 6 devices from 3 hospitals and tested for VTEC by using rapid PCR technique were positive (Figure 1). While only 33.3% (2) of swabs tested by conventional PCR technique showed positive detection for VTEC. (Table 2).

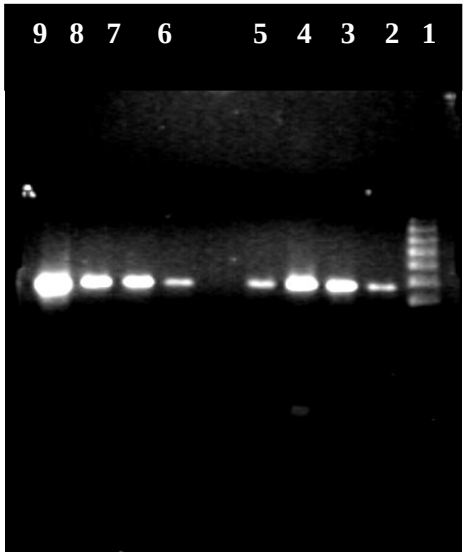

Fig. 1: Rapid PCR- products of Vt-2 gene on 1.2 % agarose gel. line (1) Molecular markers (100 bp) and (2-9) lines represente the samples PCR products dublicat .

Table 2: The results of detection of Verotoxic*Escherichia coli* by PCR technique.

Site of isolation	No. of sites	No. of devices	Percentage of identified VTEC by rapid PCR	Percentage of identified VTEC by conventional PCR
University	2	6	0%	0%
Hospital	3	6	66.6 %	33.3%

The current results recorded that the surfaces of all fingerprint devices in the two universities included in this study were free of *Verotoxic Escherichia coli* comparison with samples which collected from hospitals fingerprint devices, which were contaminated with VTEC. That indicate that the nature of work place play an important role in determination the probability of infection transfer. This is agree with many of studies[9,15] which reported that most bacterial infection associated with contaminated inanimate objects(environmental surfaces), where bacteria can be transfer by direct or indirect contact. The transmission of these bacteria from inanimate objects to human is occur commonly via hand contact with the surface and the risk of transmission depend on the numbers of transferred bacteria and other expected conditions such as social level, geographic location and epidemiological factors. Crowded places such as hospitals, universities and airports gives high chance to get such transmission.

Number of recent studies reported that the fingerprinting process as used now play an important role in transmission of viral infection such as *hepatitis A virus*, *rotavirus*, *norovirus* , *influenzavirus*, *etc*. and enteropathogenic bacteria which cause disease with low infectious doses for example: *Verotoxic Escherichia coli* and *Shigella dysenteriae*[16,17]. These studies agree with the results of current study which succeeded in isolating *Verotoxic Escherichia coli* from the surface of fingerprint devices. The nature of work place is also responsible for risk of microbial infection transmission and hand hygiene is important but insufficient to minimize the chance of this transfer. So cleaning and disinfecting of environmental surfaces is fundamental in

reducing transmission of infections [18]. Application of ethanol hand gel 85% or isopropyl hand rub 70% reduces the risk [9].

Conclusions:

The fingerprinting process as used now is responsible for risk of *Verotoxic Escherichia coli* transmission in crowded places such as hospitals, universities and airports. So need periodic evaluation for the surfaces of fingerprint devices to make sure they are free of pathogens. We recommend by using the rapid PCR technique for detection of VTEC because it easy, take short time (only number of hours), do not need additional steps or kits for DNA extraction and it have low cost comparison with other conventional methods that have high cost of laboratory diagnosis procedures,.

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