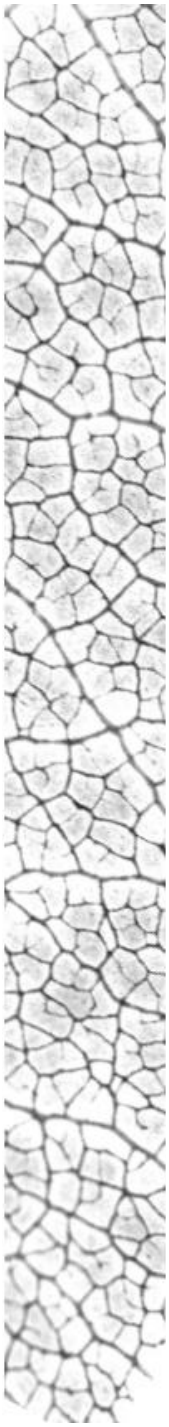

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Molecular investigation of an unusual bacteria causing severe UTI among public water closet users: Mechanisms of characterization and Engineering solutions

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Abstract
Urinary tract infections (UTIs) compose one of the most common community-acquired infections, where Escherichia vulneris does not signify a pathogenic role in it. Nevertheless, recent studies indicate that insufficient water closets play a vital role in the implications of disease transmission and health problems for all attendees. Besides, the defective water closets at the educational facilities with insufficient access to clean toilets could bring harmful health effects such as severe urinary tract infections for both staff members and students due to their exposure to bioaerosol infections which are transmitted easily in dirty public water closets. Thus, this study designed to assess the prevalence of urinary tract infections among defined employees with the estimation of the role of individual water closets in the incidence of microbial etiology.
The Results show significant prevalence of UTIs recorded among employees of both sexes working at an educational facility and sharing the same unisex water closets during their daily-worktime. The causative pathogen isolated from both positive UTI urine samples and water closets was E.vulneris profiled by API 20E, identified by 16s rRNA gene sequence and the production of heat-labile and hemolysin toxins molecularly characterized by RT-PCR. The water closets indoor air quality and performance evaluated with sensors to plot the link of the UTI microbial etiology.
From this study can be Concluded a Significantly prevalent upper and lower UTI cases encountered amongst employees of an educational facility where Escherichia vulneris was the main invading pathogen. Isolated strains of E.vulneris revealed a broad spectrum of antibiotic resistance and susceptibility to Cephalosporins and Ciprofloxacin only, the gene sequence of 1400bp of the 16s rRNA, and the production of hemolysin (hlyA 1177bp), and heat-labile (LT 508 bp) toxins. Besides, the inadequacy of the water closet at the workplace imposes an extra burden on the health of the employees as it might promote many community-acquired diseases.

Keywords: molecular characterization, E.vulneris, employees, educational facility, Water Closets, UTI, RT-PCR

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INTRODUCTION

Community-acquired UTIs are associated mainly with substantial morbidity and mortality rates in the general population and impose a substantial financial burden on society (Fiorentino et al. 2019; Mohiuddin, 2019; Amend et al. 2020). Meanwhile, Escherichia vulneris is an opportunistic human pathogen that has been isolated from many sources, but without clinical reports of human urinary tract infections worldwide (Horii et al. 2001; Nimri, 2010; Linhares et al. 2013). It is a Gram-negative bacillus that can colonize the respiratory tract, female genital tract, urinary tract, and stool (Pien et al. 1985; Spaulding and Rothman, 1996; Stock and Wiedemann, 1999). However, its tendency for wounds led to the name of vulneris-Latin for “wound” (Pien et al. 1985), where reports recognize this bacterium as a colonizer, not a precedent in prevalent infections such as UTI (Senanayake et al. 2006; Mohanty et al. 2005; Jain et al. 2016).

On the other hand, numerous researches had demonstrated that improper public water closets constitute the vital, missing link between the emergence of new bacteria and disease transmission among the users (Roberts, 2003; Hung, 2004; Cunningham and

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Greed, 2005; Paulina, & Hammed, 2018).Moreover, other studies exposed many unaware people of the risk of air-borne microorganisms present in the water closets and the impact of the spread of infections within their society (Fowler, 2001; Hanson et al. 2004). Conversely, some educated people tend to avoid using smelly, dirty, and inappropriate water closets at their workplaces, creating some other consequences on their health (Peyrat et al. 2002; Parazzini et al. 2003). Alternatively, investigators indicated that adequate and well-designed public water closets positively contribute to people's health and quality of life where the insufficient water closets could facilitate the direct spread of infections from one toilet user to another and the rise of an innovative bacteria (Peyrat et al. 2002; Parazzini et al. 2003; Mackenbach, 2003). Nevertheless, the impact of inadequate public water closets had captured the interest in only a few studies where the education facilities were almost missing from many pieces of literature, particularly in Iraq. Therefore, this study aimed to meet the following objectives:

1- To evaluate the prevalence of urinary tract infections among defined employees, and to outline the infecting microorganism.
2- To allocate the source of the infection.

MATERIAL AND METHODS

Participants enrolled in the study

A total of (100) employees in the age range of (25-60 years) from both sexes participated in this screen for UTI during the period from September 2019 till February 2020. They all worked at Building-R educational facility, and (80) of them use the unisex water closets during their work-daytime (2-3 times/day). The rest of them (n=20) never use the workplace-sanitary facility at all and were considered as (Controls). Males and females ratioed (1:1) in each study group.

Water Closet facilities targeted by the study

Four Unisex water closet sectors specified for employee's occupant within the first and second floor of the educational facility (Building-R) were the subject of this study. They carried the coding numbers of (WC1/C2B07-F1R8, WC2/C2B07-F1R7, WC3/C2B07-F2H2, and WC4/C2B07-F2H3), respectively, according to "The National Classification of the Quality of Iraqi Universities 2019). Investigated water closets situated in the area of (40 m^2)/sector. Each sector contained five cabinets with eastern sanitary seats.

Study Design

This study designed to include two levels of investigations; the first part intended to evaluate the prevalence of UTI among the employees, and defining the microbial etiology of the infection. While the second part of the study proposed to decide the role of the effect of water closets situated at the workplace on employee's health.

The evaluation of UTI prevalence among employees

1- Data collection: Data collection conducted via a questionnaire fully disclosed to this study involving the participant's name, age, gender, residence, the code of the WC sector they use, number of voids/day, their perception of the sanitary sector, past medical history, and any unusual, symptoms.

2- Urinalysis test

1. Urine collection: Clean- Catch midstream fresh urine samples of (30 ml) collected randomly from (100) employees during their work-daytime after voiding in the water closets investigated. Pregnant employees, diabetic, high blood pressure employees were not involved. All participants had signed out written consent, and urine samples collected into sterile disposable containers for analysis according to the standard procedure cited by (Memişoğulları et al. 2010).

2. Urine evaluation: The evaluation performed using reagent test strips (Mission® Urine Reagent Stirps /USA) and result interpretation implemented according to the reference sheet of the strip manufacturer.

3. Microscopic examination: Ten milliliters of each urine sample centrifuged for five minutes at 3000 rpm. A drop from the sediment mixed with drops of sterile normal saline and examined directly under the (40X) power of a light microscope for the presence of (crystals, red blood cells, leukocytes, and bacterial cells). Further, suspected UTI samples inoculated on culture media and subjected to further microbiological analysis.

Cultivation of the infecting microorganisms

Different types of culture media, including (Nutrient agar, MacConkey, and blood agar Himedia /India), used for the cultivation of the suspected positive urine samples beside the inoculation of swabs collected from different locations in the target water closet sectors. Media prepared according to the instruction of the manufacturer company, distributed onto Petri dishes and incubated overnight at 37 ℃ before sample collection.

Gram's staining: This procedure followed the reference sheet supplied by the manufacturing company of the Gram stain Kit (Himedia/India). Slides examination under the (100X) objective of the light microscope designated the stained deep blue bacterial isolates as Gram's positive and the bacterial isolates which stained with pink to red as Gram's negative. Slides photographed with a mobile camera.

Analytical profile index system for identification of Enterobacteriaceae using (API 20E kit): This procedure followed the instructions supplied by the manufacturing company (API® 20E Kit/BioMeriux/ France) and strips recorded by referring to the "Reading Table" where strain identification achieved with the numerical profile

Kirby-Bauer's disc diffusion method: Mueller-Hinton agar plates inoculated with (1 ml) of bacterial

Table 1. Primers Design for the Molecular Identification of Escherichia vulneris (E &W) isolated strain's 16s ribosomal (rRNA) gene sequence banding pattern

Isolated Bacterial Strain	Primer Sequences (5′ to3′) For PCR Amplification	Gene Bank No.	16s rRNA (bp)	Type of DNA Marker
Escherichia vulneris (E &W)	27F: Forward Primer 5′-AGAGTTTGATCCTGGCTCAG-3′ 1492R: Reverse Primer 5′-GGTTACCTTGTTACGACTT-3′ BIO-RAD/USA	NCBI/ KX357823/ USA	1400 bp	Lambda DNA/ EcoRI/ HindIII digest 21,226 bp-564 bp Jena Bioscience GmbH/ Germany

Table 2. Primers Design for the Molecular screening of Virulence Markers of Escherichia vulneris (E and W) isolated strains banding patterns

Isolated Bacterial Strain	Primer Sequences (5′ to3′) used For Virulence Markers /PCR Amplification	Virulence Markers Banding pattern	DNA Marker
Escherichia vulneris (E &W)	LTF: Forward Primer 5′- GGCGACAGATTATACCGTGC-3′ LTR: Reverse Primer 5′- CCGAATTCTGTTATATATGTC-3′	508 bp	Lambda DNA (100 bp)/ Jena Bioscience GmbH/ Germany
	STF: Forward Primer 5′- TTAATAGCACCCGGTACAAGCAGG-3′ STR: Reverse Primer 5′-CTTGACTCTTCAAAAGAGAAAATTAC-3′	255 bp	
	hlyAF: Forward Primer 5′- AACAAGGATAAGCACTGTTCTGGCT-3′ hlyAR: Reverse Primer 5′- ACCATATAAGCGGTCATTCCCGTCA-3′	1177 bp	

suspension to be tested before placing the antimicrobial disc (30 µg BIO-RAD/France) on the agar surface. Plates incubated at 35 °C for 20 hrs, and the millimeters of the clear zones of growth inhibition around the disks, measured using precision calipers. The zone diameter for individual antimicrobial agents determined according to the interpretation table of the CLSI of (Brenner et al. 1982).

Molecular Identification of the 16s ribosomal (rRNA) gene sequence for Escherichia vulneris (E&W) isolated strains: Those reactions followed the protocols cited by (Jain et al. 2016) used for the identification of the16s ribosomal (rRNA) gene sequence of both E. vulneris strains isolated earlier from employees and water closets. Single isolated colonies from overnight E. vulneris (E and W) cultures picked up with sterile micropipette tip and added to Colony PCR reaction. Each PCR reaction has contained (5 µl) of PCR buffer (1 unit) of Taq DNA polymerase (BIO-RAD/USA), (25 pmol) of forward and reverse primers as described in **Table 2**. PCR amplification cycles performed at 94 °C for 45 s, 55 °C for 60 s, and 72 °C for 60 s, for the elution of (1400 bp) 16s rRNA ribosomal gene sequence band. PCR products run on (1.8%) agarose gel electrophoresis, visualized under UV Transilluminator, and pictured using a gel documentation system.

The Molecular Screening of Virulence Markers of E. vulneris (E &W) isolated strains: The isolated strains of E. vulneris (E and W) screened for virulence markers encoding enterotoxins heat-labile (LT), heat-stable (ST), and hemolysin (hlyA) associated with sepsis. Genomic DNA extracted using a kit according to the instructions of the manufacturer-company (ThermoFisher Scientific/USA) from an overnight grown culture in tryptic soy broth (Himedia/India) and used as the template. Six forward and reverse primers (BIO-RAD/USA) for the identification of the three toxins consumed in the reaction, as their design is shown in **Table 2**. The Real-time PCR reaction performed using a predefined program with an initial denaturation at 95°C for 5 minutes of 35 cycles of denaturation at 95°C for 30 seconds, as cited by (Jain et al. 2016).

RT-PCR products run on (1.8%) agarose gel electrophoresis, visualized under UV Transilluminator, and pictured using a gel documentation system.

The valuation of Water Closets (IAQ) & Performance as Health Facilitator according to Standard Measurements: In this study, technical engineering analysis applied to evaluate water closets suitability as health facilitators in comparison with the global standard criteria for sanitary systems (IAQ) according to (Cunningham and Greed, 2005; Rahman et al. 2014). The analysis steps were particularly planned for this study as follows:

1- Determining the adequacy of minimum indoor lighting with the air quality, including the parameters of (airflow rate CO_2 and SO_2) measured by using types of equipment placed vertically at the height of 1.2 m from the floor during the measurement periods.

2-Subsequently, the flow rate is converted into ventilation rate to determine the adequacy of indoor air distributed for comparing with CIBSE standards and IAQ.

The ventilation rate in the water closets calculated using the following equation:

$V_r = Q$ / person

Where: Vr = Ventilation rate (l/s/p)

Q = Airflow rate (m^3/s)

3. The adequacy of the ventilation system determined by calculating the number of working air exhausters per water closet section.

4. The floor, walls, and ceiling quality determined by being free from dirt, stains, molds, and peeling paint.

5. The determination of the performance of the sanitary pipes at each cabinet.

6. Evaluation of water and soap adequacy in the water closets.

7. Estimating the rate of health facility cleaning per day.

8. Plotting the link between the water closet performance, staff perception, and the effect on their health parameters.

Statistical analysis

The statistical analysis of the study data obtained conducted using SPSS program Ver.10 for Windows and percentages calculated using The Two-Sample t=test. The p-value of <0.05 considered a statistically significant difference, according to (Asmi et al. 2012).

Table 3. The percentage of urinary tract infection symptoms between the total of one hundred employees of Building-R enrolled in the study

Symptoms of Urinary Tract Infections	% of Positive Employees[a]	% of Positive Controls[b]
Upper UTIs %[c]	33.6	0.4
Fever/chills	18	0
Nausea/vomiting	9	1
Flank pain	28	0
Abdominal bloating/flatulence	29	1
Lower UTIs %	88.8[d]	0.6
Nocturia	32	1
Dysuria	8	1
Urinary incontinence	8	0
Pain/burning during urination	27	0
Lower abdominal pain/heaviness	36	1

[a] Abbreviations: Male & female WC- users of Building -R employees; [b] included: Male & female WC- not users of Building-R employees; [c] Percentages calculated using The Two-Sample t=test; [d] individual positive employees had symptoms of both types of UTI.

Table 4. Screening results of the Urinalysis test Reagent-strip method of urine samples collected from eighty participants suspected with urinary tract infection enrolled in the study

Reagent Strip Parameters	% Positive Employees n = 80
Leukocytes (Leu/ µL)	70-500 (100%)
Nitrite (NIT) > 20ppm	20-50 ppm (100%)
Urobilinogen (mg/dL)	1-4 (21%)
Protein (mg/dL)	0.15- 1.0 (12%)
pH	7-8 (100%)
Blood in Urine (Ery/µL)	5 (87%)
Specific Gravity (SG)	1.010-1.020 ((96%)
Ketone (mg/dL)	0.5- 1.5 (14%)
Bilirubin (mg/dL)	1(18%)
Glucose in Urine (mg/dL)	100 ±10 (5%)

*Values estimated according to the Interpretation Table of Mission® Urine Reagent Strips/ ACON/USA.

Table 5. Bacterial types isolates from positive urine samples (n = 240) collected from urinary tract infected employees of both sexes at different intervals during the study described in percentage/urine sample

Types of Gram's negative bacterial Isolates / Urine sample	%of bacteria / 240 Urine sample		Types of Gram's positive bacterial isolates/ Urine sample	%of bacte Urine sa
	Female n=120	Male n=120		Female n=120
Escherichia vulneris	82(68.33)	78(65.0)	*Staphylococcus epidermidis*	31(25.83)
Escherichia coli	62(51.67)	47(40.0)	*Staphylococcus aureus*	22(18.33)
Klebsiella pneumoniae	17(14.16)	20(16.67)	*Enterococci spp.*	1(0.83)
Enterobacter spp.	10(8.3)	6(5.0)		

*urine samples collected three times from UTI patients at separated intervals to rule-out bacterial

RESULTS

The total of (100) employees subjected to extensive questions within the study questionnaire and the results shown in **Table 3**. Consequently, the results of **Table 3** encouraged the assumption of the incidence of urinary tract infections among the employees who used the water closets at their workplace (Building -R). Thus, all of the study participants performed the urinalysis test, and the results of strip reagent revealed in **Table 4**.

Consequently, the results of **Table 3** encouraged the assumption of the incidence of urinary tract infections among the employees who used the water closets at their workplace (Building -R). Thus, all of the study participants performed the urinalysis test, and the results of strip reagent revealed in **Table 4**.

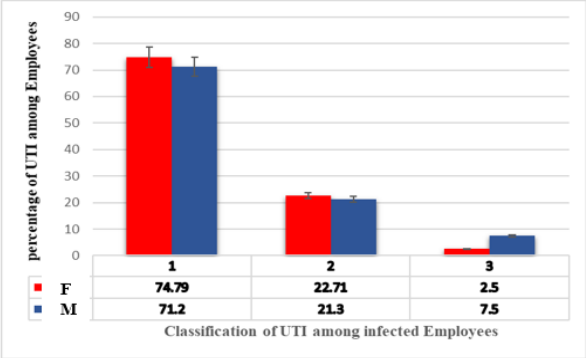

Fig. 1. The classification of UTI patients enrolled in the study according to clinical presentation and severity level of infection displaying Cystitis (Legend 1), pyelonephritis (Legend 2), and urosepsis (Legend 3)

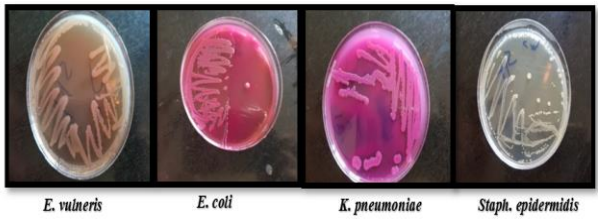

Fig. 2. Examples of bacterial colonies growing on different culture media isolated from positive urine samples from UTI patients enrolled in the study

Continuously, A statistical analysis implemented between the numbers of the UTI patients of this study and the severity level of the symptoms declared in **Table 5**, which signified the percentage of the clinical presentation of positive results declared in **Fig. 1**.

Accordingly, the positive leukocytes and nitrite urine samples with a bacterial count over (10^5 UFC/ml) were cultivated according to the standard procedure on different identification culture media and some photos of positive cultures growing on MacConkey and nutrient agar shown in **Fig. 2**.

Subsequently, the microscopical examination of the isolated bacterial colonies revealed two Gram's positive isolates of Staphylococcus aureus and Staphylococcus epidermidis with the predominance of a novel Gram's negative rods of Escherichia vulneris. Thus, all bacterial isolates additionally examined by subjecting the purified colonies to the (Analytical Profile Index System for Identification of Enterobacteriaceae using API 20E Kit). Then, the results of the API 20E profiles equated with the results of the positive urine sample and the incidence rate of the isolated bacteria in the samples exposed in **Table 5**.

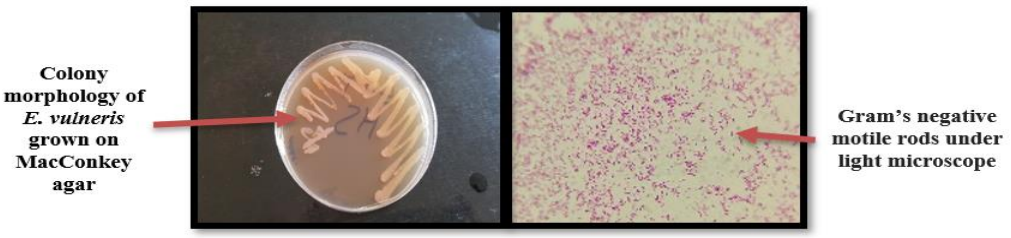

Fig. 3. Colony morphology and Gram's staining of Escherichia vulneris isolates obtained from the four water closets investigated in this study

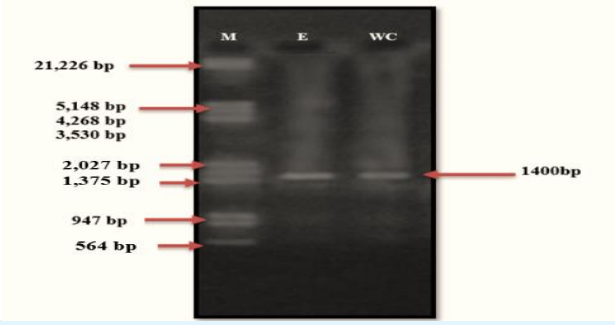

Fig. 4. Gel picture of Colony-PCR products of 16s rRNA gene sequences of two E. vulneris strains amplified with 27F, and 1492R primers run on (1.8%) agarose gel, visualized under UV Transilluminator and pictured using a gel documentation system. Lane 1, gene sequence of E. vulneris E and Lane 2, gene sequence of E. vulneris W. M, Marker λ DNA EcoRI and HindIII digest

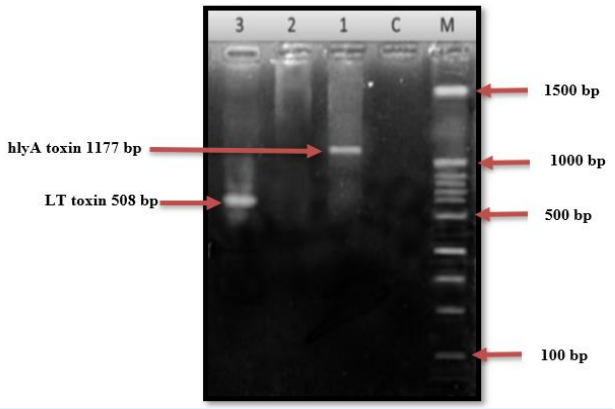

Fig. 5. Gel picture showing Escherichia vulneris positive for hlyA toxin (1177 bp, Lane 1); negative for heat-stable (ST) toxin (Lane 2) and positive for heat-labile (LT) toxin (508 bp, Lane 3); heat-stable (ST) toxin. PCR mixture without the template as a negative control (Lane C); 100-bp ladder DNA marker Lane M). RT-PCR products were run on 1.8% agarose gel and visualized under UV Transilluminator and pictured using a gel documentation system

Results presented in **Table 5** showed a predominance of the bacterium E. vulneris in (68.33-65%) of the urine samples where the growth pattern and Gram's staining is shown in **Fig. 3**.

The fermentative E. vulneris has grown on MacConkey agar at 37 °C and colonies, as presented in the photos of **Fig. 3** looked generally smooth, creamy with shiny surfaces and low convex. Besides, Gram's staining of the isolated colonies revealed Gram-negative, none-spore forming motile rod-like (bacilli).

Also, the antimicrobial susceptibility of (160 isolates of E. vulneris E) and (40 isolates of E. vulneris W) tested for susceptibility to (16) antibiotics by the Kirby-Bauer's disc diffusion method. All strains were uniformly susceptible to (Cephalosporins and Ciprofloxacin). In contrast, most of the E. vulneris strains were resistant against (Amikacin, Carbenicillin, Clindamycin, Chloramphenicol, Penicillin, nitrofurantoin, Gentamicin, Kanamycin, polymyxin B, Tetracycline, Trimethoprim and, Erythromycin) according to the recommendations of the National Committee for Clinical Laboratory Standards cited by (Shobrak and Abo-Amer, 2014).

Still, the isolated E. vulneris strains of this study showed innovative results of antibiotic susceptibility when compared to the results of antibiotic susceptibility asserted by other researches, where their isolated strains showed susceptibility to all of the antibiotic types mentioned earlier according to (Kresken et al. 2016; Terlizzi et al. 2017).

Accordingly, two amplicons of E. vulneris (E and W) tested with Colony-PCR for the molecular identity of the (1400 bp 16s rRNA gene sequence of Gram's negative E. vulneris) using two universal primers (a forward primer 27F and a reverse primer 1492R. Both primers reacted with the strains at an annealing temperature of 50°C. Then, the products of PCR ran on 1.8% agarose gel and photographed. The amplicon of around 1400 for both isolates was detected and photographed, as results demonstrated in **Fig. 4**.

In accordance, the isolated strains in this study showed (100%) identity with the E. vulneris strain isolated formerly in 2016 from an infant in India with severe diarrhea and sepsis (Accession No. KX357823 in NCBI bank), cited by (Jain et al. 2016). Moreover, molecular screening of the virulence markers encoding hemolysin (hlyA), heat-stable (ST), and heat-labile(LT) enterotoxins associated with sepsis also carried out, and results are shown in **Fig. 5**.

Results of the molecular characterization of E. vulneris isolated from water closets causing severe UTI among employees had highlighted the probability of the

Fig. 6. Photos of implementation inaccuracies, inadequacy, sparse sanitary hygiene practice Water Closets of an educational facility, where; Fig. (6a) closed air exhauster fan, Fig. (6b) inaccurately implemented air exhauster Fan, Fig. (6c) air exhauster fan closed with a boiler, Fig. (6d) peeling ceiling and Fig. (6e) pipe blockage with a sanitary pad

Table 6. Performance adequacy according to global /Standard engineering implementations of the four water closets situated at Building R of the educational facility

Standard WC Parameters Measures	Performance of the Four Water Closets/ Building R				Total scores /Standard Measures
	WC1 C2B07-F1R7	WC2 C2B07-F1R8	WC3 C2B07-F2H2	WC4 C2B07- F2H3	
Minimum Lighting Indoor (300*Lux*)	150 *Lux*	110 *Lux*	50 *Lux*	50 *Lux*	Failed
Airflow rate 1.5-0.5m³/s	0.45 m³/s	0.422 m³/s	0.463 m³/s	0.055 m³/s	Failed
SO_2 Max. TLV 0.4 ppm	0.75 ppm	0.53 ppm	0.58 ppm	0.69 ppm	Failed
CO_2 ppm	75,000	55,000	42,000	80,000	Failed
Ventilation rate (*F*) Minimum 629 l/s/p	620 l/s/p	600 l/s/p	402 l/s/p	321 l/s/p	Failed
Mechanical Ventilation system (Exhaust Fan adequacy)	Closed[a]	Inaccuretly implemnted[b]	Closed[c]	Dislocated	Failed
Floor /Walls quality	Dirty/stains	Dirty/stains	Dirty/stains	Dirty/stains	Failed
Ceiling quality (Clean no paint peeling)	Peeling paint[d]	Peeling paint	Peeling paint	Peeling paint	Failed
Sanitary pipes quality (break/blockage)	Blockage /1 seat	Broken pipes/ 2 seats	Broken pipes/ 2 seats	Blocked pipes / 3 seats[e]	Failed
Water adequacy (availability of water)	Two times/week	Two times/week	Two times/week	Two times/week	Failed
Soap adequacy (No. of soap/Washing basin)	1/5 washing basin	None	None	One liquid/ 5 washing basin	Failed
Facility Cleaning Rate (FCR/ day)	Once/Week	Once/Week	Once/three weeks	Once/month	Failed

* [a,b,c,d,e,] Performance failures featured at Figure (6).

inadequacy of these sanitary facilities for the proper performance as a health facilitator. Therefore, all of the water closets utilized by the infected employees subjected to extensive engineering queries according to the (IAQ) parameters and Global Standards disclosed in **Table 6** with the performance results of the water closets.

Results presented in the table above demonstrated deficient indoor lighting measures of (< 150 Lux) in all water closets in addition to very low airflow (0.45- 0.055 m^3/s. The SO_2 levels were also measured and significantly elevated levels of (0.53-0.75 ppm) more than the recommended TLV maximum acceptable levels of (0.4 ppm). This elevated level of SO_2 levels was probably due to the low ventilation rate with the inadequate ventilation system as the photos gathered in **Fig. 6** through more light on the poor performance of the four water closets situated in Building-R.

The photos above revealed a window-mounted air exhauster fan that is always off in the water closet coded (WC1/C2B07-F1R7 (**Fig. 6a**), or a fan inaccurately implemented in the water closet coded (WC1/C2B07-F1R8 (**Fig. 6b**) or a closed one by a boiler in the water closet coded (WC1/C2B07-F2H2 (**Fig. 6c**). Besides, the ceilings of the water closets were peeling paint (**Fig. 6d**), and some of the sanitary pipes of the eastern seats blocked with a sanitary napkin or menstrual pads (**Fig. 6e**) in almost all water closets investigated.

DISCUSSION

An average human being uses a water closet in about 2,500 times or what equals three years of his life (Muhamad-Darus *et al.* 2011; Ohagim *et al.* 2017; Sunarsa and Andiani, 2019). Besides, many people spend most of their work time indoors, where they frequently share public water closets without realizing their role in disease transmission, mainly urinary tract infections (Fowler, 2001; Hung, 2004; Hanson *et al.* 2004). As a matter of fact, in developing countries, urinary tract infections are estimated to affect over 150 million people annually, most of them acquired from insufficient water closets

(Wilson *et al.* 2000; Gupta *et al.* 2001).In this regard, this study has been suggested to verifying the validity of employee's complaints about their workplace health facilities' role in disease transmission.

Consequently, the results of the questionnaire suggested the probability of upper and lower urinary tract infections among the employees share the water closets at their workplace, and since (UTI) has been defined as the diverse spectrum of conditions causing multiple clinical complaints (Amatya et al. 2016). Thus, in order to make the right judgment about the type of infection, a conclusive diagnostic test performed. Presently, the urinalysis test strip method is considered as a valid rule-out test for people with suspected UTI since its crucial advantage is the combination of leukocyte and nitrite negative results exclude UTI from the sample as cited by (Sunarsa and Andiani, 2019). Successively, data recorded in **Table 5** signaled a total of (100%) of the participants with positive test results for both leukocytes and nitrate reduction, respectively confirming the prevalence of UTI among male and female employees at all age groups and the presence of large counts bacterial cells in their urinary tracts.

However, the results obtained in **Table 5** agree with the results of previous researches where they had regarded urinary tract infections (UTIs) are one of the most common bacterial infections in humans where it

affects over (40%) of women and (20%) of men during their lifetimes (Magliano *et al.* 2012). Nevertheless, urinary tract infection is considered by many researchers as the most critical endemic disease affecting humans as one of the main causes of reproductive and kidney failures, general health complications, and reduction of the life expectancy (Bartoletti et al. 2016). Moreover, since UTI has been classified as upper UTI and lower UTI, but sometimes it involved both; thereby, current classification in 2019 depended on the symptoms with the anatomical part involvement and classified UTI into pyelonephritis (infection of the upper UTI) or cystitis (infection of the lower UTI) or urosepsis (Magliano *et al.* 2012; Kot, 2019).

Reviewing the results revealed in **Fig. 1** bare a significant increase ($p<0.05$) in the incidence rate of cystitis (lower urinary tract infections) in (74.78% and 71.2%) among the female and male employees, respectively. While the pyelonephritis (upper urinary tract infections) results of both genders were close, showing the incidence rate of (22.71% and 21.3%). Whereas three males and one female employee showed urosepsis, and they were formally sent to the hospital for urgent treatment.

Though, the percentage of female cystitis recorded in **Fig. 1** might agree with many types of research that considered lower UTIs in healthy non-pregnant women is very common and is not treated in 20–80% of cases. Nevertheless, the significant increase ($p<0.05$) in the incidence rate of cystitis (71.2%) among men employees was *not* an expected result since women, mainly those aged 16–64 years, are significantly more likely to experience UTIs than men as Hummers-Pradier *et al* in 2004 declared. Also, researchers on (UTI) has always focused on women due to a much higher incidence wherein men it is acceptable with more complicated disease and a higher risk of complications (Magliano *et al.* 2012; McLellan and Hunstad, 2016). Therefore, the urosepsis cases were higher in males than female employees.

Still, the results declared in **Fig. 1** revealed an agreement with the results obtained earlier in **Table 4** where a significant number of employees demonstrated a prevalence of (66.4%) of lower UTI with (33.6%) of upper UTI excluding the urosepsis patients who demonstrated mixed symptoms of both UTI types.

On the other hand, urinary tract infections (UTIs) are among the most common community-acquired infections associated with increased morbidity and mortality rates in the general population (Hummers-Pradier *et al.* 2004). Therefore, the above-attained results impose an urge to investigate the microbial etiology of infectious organisms. Consequently, a study published in 2015 has suggested that the cultivation of urine samples should follow the positive urinalysis test results for the proper identification of the microorganisms (Memişoğulları *et al.* 2010; Kot, 2019). Thus, positive leukocytes and nitrite urine samples with a bacterial count over (10^5 UFC/ml) were cultivated according to the standard procedure on different identification culture media. Positive cultures stained with Gram's stain and examined under the light microscope. Results of microbiological identification of the infecting bacteria did not agree with the results cited previously by other authors where they had asserted the prevalence of *E.coli* in the microbial etiology of UTIs in 50–80% of cases (McLellan and Hunstad, 2016; Kresken *et al.* 2016).

At the same time, the utility environment also was evaluated, and the formerly isolated *E. vulneris* from employee's urine samples also emerged as prevalent bacterium as revealed by the API 20E profile.

To our knowledge, this is the first defined case of *E. vulneris* associated with community-acquired urinary tract infections among people sharing the same water closets. Though, many researchers had considered that *E. vulneris* as an opportunistic human pathogen that initially colonizes wounds (Pien, 1985; Jain *et al.* 2016). Later, this pathogen has been identified as the sole pathogen in various infections such as bacteremia from an infected intravenous catheter (Spaulding and Rothman, 1996), meningitis (Mohanty *et al.*2005), urinary sepsis (Awsare and Lillo, 1991) osteomyelitis from a foreign body (Levine and Goldberg, 1994).

Thus, in order to comprehend the association between the two isolates strains of *E. vulneris* (i.e., *E. vulneris* E and *E. vulneris* W) and their starring role in increasing the incidence rate of urinary tract infections among employees. The antimicrobial susceptibility of the isolates tested, and the isolated *E. vulneris* strains of this study showed innovative results of antibiotic susceptibility when compared to the results of antibiotic susceptibility asserted by other researches, where their isolated strains showed susceptibility to all of the antibiotic types mentioned earlier as cited by (Kresken *et al.* 2016; Terlizzi *et al.* 2017).

Besides, other researches have relied on the 16s rRNA gene sequence analysis for the identification of novel pathogens with new emerging sort of infections and antibiotic resistance isolated from sophisticated microbial inhabitants (Jain *et al.* 2016)**.** Accordingly, the isolated strains in this study revealed (100%) identity with the *E. vulneris* strain isolated formerly in 2016 from an infant in India with severe diarrhea and sepsis (Accession No. KX357823 in NCBI bank). Moreover, the results of the molecular characterization of *E. vulneris* isolated from water closets causing severe UTI among employees had highlighted the probability of the inadequacy of these sanitary facilities for the proper performance as a health facilitator.

Likewise, unisex-water closets, the low cleaning rate of the sanitary facility, water deficiency, improper airflow and, inadequate ventilation system, together with irresponsible hygiene behavior (throwing sanitary napkin

in the eastern seat drain nozzle), had combined to create foci of an opportunistic, toxin-producing, multi-drug resistant wound inhabitant bacteria to grew in these utilities. The same results have confirmed this conclusion, as previously demonstrated by Kresken *et al.* that a workplace is a public facility, which is frequently used by people and located indoor. Therefore, maintaining good air quality in the toilet is essential in order to keep it hygienic and sanitary for employees.Moreover, exceeding the TLV of SO_2 and CO_2 above the acceptable values of (IQA) was also determined by (Muhamad-Darus *et al.* 2011; Linhares *et al.* 2013; Ohagim et al. 2017) as the inadequacy of air velocity/ventilation failed to dilute the indoor SO_2, where the inhalation of this pollutant is associated with increased respiratory illness, wheezing fits, asthma, and prolonged health effects on the elderly, and those with cardiovascular or chronic lung disease.

CONCLUSION

1. Significantly prevalent upper and lower community-acquired UTI encountered amongst many male and female employees of an educational facility sharing the same water closets at their workplace.
2. Employees revealed cystitis, pyelonephritis, and urosepsis associated with *Escherichia vulneris* as the main etiological factor of the infection.
3. Isolated strains of *Escherichia vulneris* revealed a broad spectrum of antibiotic resistance and susceptibility to only a few.
4. Colony-PCR revealed the gene sequence of 1400bp of the 16s rRNA in *E. vulneris* strains isolated from employees and water closets, and RT-PCR results confirmed the production of hemolysin (hlyA 1177bp), and heat-labile (LT 508 bp) toxins.
5. Unisex-water closets at workplaces with low-performance values and irresponsible sanitary hygiene behavior imposed an extra burden on the health of the employees as it might promote UTI.

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