ISSN 1314-6246 Dururu *et al.* *J. BioSci. Biotechnol.* **2024**, 13(1): 35-41

RESEARCH ARTICLE

Mathias Dururu
Petros Muchesa
Farisai Chidzwondo
Rumbidzai Mangoyi

Water and surfaces as potential sources of pathogenic bacteria in Harare hospitals' intensive care units

Authors' address:
Department of Biotechnology and Biochemistry,
University of Zimbabwe,
P.O. Box MP 167, Mt Pleasant,
Harare,
Zimbabwe.

Correspondence:
Rumbidzai Mangoyi
Department of Biotechnology and Biochemistry,
University of Zimbabwe,
P.O. Box MP 167, Mt Pleasant,
Harare,
Zimbabwe
Tel.: +263773650560
e-mail: rrumbie.2000@gmail.com

Article info:
Received: 21 March 2024
Accepted: 14 May 2024

ABSTRACT

Water and contaminated hospital surfaces have been reported as major causes of Hospital acquired (nosocomial) infections (HAIs), a significant global safety problem for both patients and medical staff. However, the frequency of Intensive Care Units (ICU)-HAIs has been reported to be at least 2-3 times greater in low- to middle-income countries, particularly in Zimbabwe, than in high-income countries. Thus, this study aimed at determining the presence of pathogenic bacteria in water and surfaces from selected high-risk areas within the intensive care units of three hospitals (A, B, and C) in Harare, Zimbabwe. Water and surface samples were collected and processed within 24 hours of collection. The water samples were tested for the presence of microbial indicators using standard methodology of microbial plating. The surface swabs were also investigated using microbial plating. The organisms that were found to be present in surface samples were then identified using biochemical tests and 16S polymerase chain reaction (16S PCR). The results showed no bacteria present in any of the water samples tested during the study. However, using morphological characteristics, six different types of bacteria were identified in surface samples. The bacteria identified to be present on surfaces were *Escherichia coli, Salmonella, Shigella, Staphylococcus, Pseudomonas,* and *Streptococcus*. Hospital A surfaces may be the major cause of some bacterial infection outbreaks, hence, there is a need for better ways of reducing contamination of surfaces in the intensive care units.

Key words: Intensive care units, water, surfaces, bacteria, biochemical tests, 16S polymerase chain reaction

Introduction

Approximately 18% of outbreaks associated with public water systems in the world are attributable to microbiological pathogens and toxic substances contaminating the water distribution networks (Bitton, 2014). The excessive development and colonisation of water distribution pipes by bacteria and other pathogenic organisms are the main cause of the decline in water quality, which can have detrimental effects on public health. The World Health Organisation (WHO) reports that at any given moment, 7% of patients in affluent nations and 10% of patients in underdeveloped countries develop at least one Hospital Acquired Infection (HAI) due to contaminated water and unhygienic handling of hospital equipment and disposals (Mathur, 2018). In high-income nations, 30% or more of hospitalised patients in intensive care units (ICUs) develop at least one HAI. However, the frequency of ICU-HAIs is at least 2-3 times greater in low- to middle-income countries than in high income (Bardossy et al., 2016; Noor et al., 2021)

In Zimbabwe, a large number of immunocompromised people are housed in public health care institutes (PHCIs) which have issues of overcrowding, lack of water and financial limitations resulting in high risks of HAIs (Harun et al., 2022). Therefore, it is crucial to regularly monitor the microbiological water quality in healthcare facilities for the sake of everyone who enters these facilities, including patients, staff, and visitors.

Thus, this study focuses on investigating the presence of pathogenic bacteria on the surfaces of the three selected Harare hospitals' Intensive Care Units, as well as in the drinking water provided to these healthcare facilities and across their distribution network. The study aims to cover support towards proper ways of preventing pathogenic bacteria outbreaks in Harare, Zimbabwe.

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20241035

Materials and Methods

Ethical and legal considerations

Senior management from each of the healthcare institutions participating in the study gave their consent for sampling to take place in the areas of their facilities.

Study sites

Three community-serving public hospitals in Harare, Zimbabwe were the sites of the study. These hospitals were named Hospital A, Hospital B, and Hospital C in this study.

Hospital A

Hospital A consists of three active in-patient ICU beds and receives patients from other wards. The ICU ward uses council water, and in cases of water shortages, it uses a large 100-litre container, as its reservoir.

Hospital B

Hospital B has a total of 8 beds in the ICU and 1 extra bed in the HDU. The ICU receives patients from medical, surgical, orthopedic, and psychiatric wards which provide in-patient facilities for around 350 patients and admit approximately 50 patients per day. The facility uses council water and has a backup borehole that can be used in water shortages.

Hospital C

Hospital C has 2 active ICU beds. The hospital also uses council water but provides borehole water for drinking to its patients.

Sampling sites

A total of 86 potential sampling places, including 29 from Hospital A, 35 from Hospital B, and 22 from Hospital C were identified (Table 1). These sampling locations included 15 municipal water flow points.

Sample collection

Every sampling location had two samples taken, thus, a total of 172 samples, 142 surface, and 30 water, in all 3 Intensive care units were collected between June and September 2023. Samples were taken in accordance with the sampling plan using sterile bottles and swabs. Swabs were utilised to gather surface samples from the surfaces in the wards. Water samples were obtained within the first one to two minutes of running tap water in 500 ml sampling bottles containing 5 mg/L sodium thiosulfate. At each sampling site, water temperature, pH, turbidity, conductivity, and total dissolved solids (TDS) were measured using a portable COMBO TESTER® (Hanna, SA) in accordance with the manufacturer's instructions. According to the manufacturer's recommendations, residual chlorine was measured using a chlorine photometer (Hanna, SA). Collected samples were transported in cooler boxes to the laboratory and processed the same day they arrived.

Sample Analysis

Microbial plating

The collected swabs and water samples were stricked onto the culture media (LB agar) and incubated at 37°C for 24 hours. The colonies were subcultured until pure colonies were obtained. The colonies were then identified using the morphological features of the bacteria.

Determination of gram negative and gram positive bacteria

The obtained pure colonies were cultured on selective media to determine the gram positive and gram negative bacteria. The selective media used were Eosin Methylene Blue (EMB) agar and MacConkey agar. The inoculated colonies were placed into an incubator for 24 h at 37°C.

Biochemical tests, for bacterial identification

Different biochemical tests were done as explained in Table 2 in order to identify the obtained and isolated colonies.

Fermentation of sugars

Determination of the fermentation of sugars, sucrose, glucose, fructose, and maltose was done to identify bacteria based on their metabolic capabilities. Change in pH and gas produced determines the metabolic profile of bacteria and hence its identity.

Bacteria identification using 16S PCR

A single colony from the selective media was added to 10 µl of TE buffer and denatured at 95°C for 10 min, then cooled at 4°C for 4 min to break the bacterial cell wall and release DNA to be used in colony PCR. Colony PCR was performed in a PCR tube which contained a 25 µl reaction of 10X Dream Taq PCR buffer (2.5 µl), 2mM dNTPs (2.5 µl), forward primer (1 µl), reverse primer (1µl), template DNA

Table 1. *Summary of sampling areas within the three hospitals.*

Sampling area	Hospital A	Hospital B	Hospital C	Total
Patient handbook	1	1	1	3
Table	3	3	2	8
Tray	3	4	2	9
Bedside Locker	3	4	2	9
Monitor	3	4	2	9
Vent	3	3	2	8
Sinks	5	6	4	15
Taps	5	6	4	15
HDU	1	2	1	4
Kitchen sink	1	1	1	3
Preparatory room	1	1	1	3

 DOI: 10.69085/jbb20241035

Table 2. *Biochemical tests for the identification of bacteria.*

Test	Procedure
Indole	Using straight sterile wire single colonies of bacteria were picked from the MacConkey sub-cultures, and the colonies suspended in peptone water. The suspension was then incubated for 24 hours. After which 500 µl of Kovacs reagent were added to the suspension. It was observed if there were any colour changes and formation of layers.
Lactose	A single colony of bacteria was selected and injected into MacConkey agar using a sterile loop. After that, plates were incubated for 24 hours at 37°C. The production of a pink color was then monitored in the cultures.
Citrate	Using a sterile inoculation loop, an isolated bacterial colony from MacConkey agar was transferred to Simmons agar. After 24 hours of incubation at 37°C with the Simmons agar, the plate was checked for any color changes.
Oxidase	Using a sterile wire, small inoculum of the test organism was taken from the Blood agar sub-cultures and smeared onto a sterile glass slide. Three drops of freshly prepared oxidase reagent were added to the slide. The slide was observed after appropriately 1 minute to see if there was a colour change taking place.
Catalase	On a clean slide, a drop of 3% hydrogen peroxide (H_2O_2) was applied using a dropper. Using a sterile inoculation loop, an isolated bacterial colony from the MacConkey agar was inserted in the drop of hydrogen peroxide to check for any effervescence in the drop.
Motility	Using a sterile straight wire, single colonies were picked from the sub-cultures of MacConkey agar and stabbed into the centre of the motility agar in a Bijoux bottle. After inoculation, they were incubated at 37°C for 24 hours. The spread pattern and colour was observed.
Coagulase	On a clean slide, a drop of 3% hydrogen peroxide (H_2O_2) was applied using a dropper. Using a sterile inoculation loop, an isolated bacterial colony from the MacConkey agar was inserted in the drop of hydrogen peroxide to check for any effervescence in the drop.
Lysine	Using lysine containing media, inoculate bacteria, incubate for 24 hours. A positive result is indicated by an alkaline slant (purple). A negative result shows no colour change.
Urease	Bacteria is inoculated into a urea-containing medium. The medium typically contains urea as the substrate and a pH indicator, such as phenol red, that changes color in response to changes in pH. A positive result is indicated by a color change from orange or yellow to pink or magenta due to the rise in pH. A negative result shows no significant color change.

Table 3. *Physicochemical parameters of the three hospitals.*

Hospital	Temp (°C)	pH	TDS (mg/l)	Residual Chlorine (mg/l)
A	14.06-28.67	6.37-9.21	9,5-196	0.01-0.37
B	17.39-32.34	7.13-8.89	108-155	0.06-0.35
C	19.0-27.0	7.13-8.46	113-150	0.01-0.36
All	14.06-32.34	6.37-9.21	9,5-196	0.01-0.37
SAZ		6.5-8.5	150-200	≤5mg/l

TDS – Total Dissolved Solids SAZ – Zimbabwean Standard

(2 µl), Taq DNA polymerase (3 µl) and nuclease free water (13 µl). The DNA was amplified with universal 16S primers, 27F, and 1492R in a thermal cycler (GeneAmp PCR System 2700, Applied Biosystems) set at 95°C for 30 seconds initial denaturing, 30 cycles of 95°C for 30 seconds denaturation, 54°C for 1-minute annealing and 68°C for 1 min extension and a final extension at 68°C for 5 min. To detect the DNA, PCR products (10 µl) were loaded onto agarose gel (1.5%) stained with ethidium bromide (6 µl) and subjected to electrophoresis in 1X Tris-acetate EDTA buffer for 40 min at 150 V and 400 mA current. The gel was visualized under UV E-gel imager. Amplicon sizes were estimated using a 100 bp DNA ladder.

Results

Physicochemical parameters

The physicochemical properties of the water at the three hospitals are shown in Table 3.

At the time of sampling, the three hospitals' water temperatures varied from 14.06°C to 32.34°C, with no noticeable differences between them. Hospital A recorded temperatures between 14.06°C and 28.67°C (mean 23.1°C), Hospital B recorded temperatures between 17.39°C and 32.34°C (mean 22.6°C), and Hospital C recorded temperatures between 19.0°C and 27°C (mean 22.6°C). The three hospitals' collected water samples had pH values ranging from 6.37 to 9.21 (mean 7.77). The reverse osmosis treatment facility, which supplies the renal unit of Hospital A, had the lowest pH ever measured (6.37). The pH ranges that

were measured at Hospital B and Hospital C were 7.13 to 8.89 (mean 7.97) and 7.13 to 8.46 (mean 7.89), respectively. The TDS levels varied from 9.5-196 mg/L (mean 125 mg/L) at Hospital A, 108-155 mg/L (mean 119 mg/L) at Hospital B, and 113-150 mg/L (mean 125 mg/L) at Hospital C. All of the levels were within reasonable limits. At the time of measurement, the residual chlorine levels in all three hospitals varied from 0.01 to 0.36 mg/L (mean 0.27 mg/L). Hospital A had a chlorine level of 0.01-0.37 mg/L (mean 0.12 mg/L), Hospital B had a chlorine level of 0.06-0.35 mg/L (mean 0.22 mg/L), and Hospital C had a chlorine level of 0.01-0.36 mg/L (mean 0.21 mg/L).

Microbiological analysis of water samples

From all the water samples that were analyzed in this research, there was no significant amount of microorganisms that grew on the LB agar.

Microbiological analysis of surface samples

All the surface samples showed the presence of bacteria. The samples were diluted from 10^0 to 10^{-4}, cultured, and colonies counted. The colony counts for all the samples are summarized in Table 4. A total of six different bacteria species were identified morphologically using phenotypic characterization. The characteristics of these different bacterial species are shown in Table 5.

Determination of gram negative and gram positive bacteria

Figure 1 shows the different bacterial colonies grown on EMB and MacConkey Agar. MacConkey is a selective and differential culture medium for gram negative bacteria hence the species that grew on this medium are gram negative and those on EMB are also gram negative. All species that did not grow on EMB and MacConkey Agar were assumed to be gram positive and Gram staining was used to verify the result.

Confirmation of the presence of E. coli on some samples

Eosin Methylene Blue agar was also used to confirm the presence of *E. coli* among the 6 obtained and isolated bacterial species. Figure 2 confirms the growth of *E. coli* as shown by the metallic green colonies obtained.

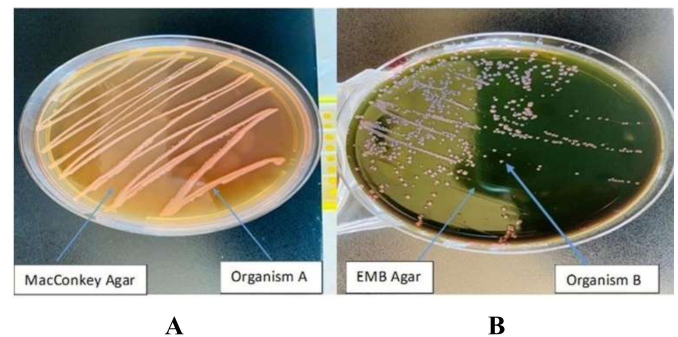

Figure 1. *Different bacterial species that grew on MacConkey Agar and EMB Agar.*

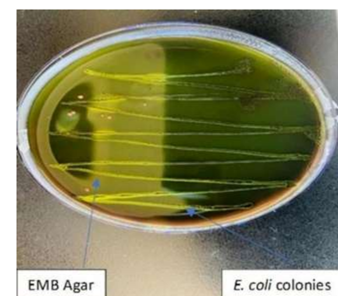

Figure 2. *E. coli cells on EMB Agar.*

Table 4. *Summary of colony count surface swabs that were diluted to 10^{-4}.*

	Hospital A		Hospital B		Hospital C	
	A	B	A	B	A	B
Patient handbook	20[a]	23[a]	42[de]	44[de]	21[e]	23[e]
Table	57[ef]	48[f]	23[af]	29[af]	25[d]	21[d]
Tray	38[aef]	42[aef]	59[af]	38[a]	45[e]	40[e]
Ventilator	26[f]	34[f]	23	20	46[f]	39[f]
Monitor	36[d]	39[d]	24[e]	19[e]	15[a]	10[a]
Bedside locker	89[ac]	67[ac]	45[bf]	50[bf]	39[af]	20[f]

A and B represent the number of two repeats that were done when collecting the samples at each hospital. [abcdef] – represent different bacterial species which were selected for identification.

Table 5. *Morphological characteristics of the identified bacterial species.*

Organism	Form	Elevation	Margin	Count/Colour
A	circular	raised	Entire	scattered
B	circular	convex	Entire	greyish
C	circular	convex	Entire	numerous
D	circular	convex	Entire	pale yellow
E	circular	convex	Entire	milky
F	circular	convex	Entire	yellowish

Table 6. *Summarized results for the biochemical tests to identify bacteria.*

Tests	Organism					
	A	**B**	**C**	**D**	**E**	**F**
Motility	+	-	+	-		-
Catalase	+	-	+	+	+	-
Oxidase	-	-	-	-	+	-
Coagulase	-	+	-	+	-	-
Indole	+	-	-	-	-	-
Citrate	-	+	-	+	+	-
Lysine	+	+	-	-	-	-
Urease	-	-	-	+	-	-
Fermentation of sugars						
Lactose	+	-	-	+	-	+
Glucose	+	-	+	+	-	+
Sucrose	-	-	-	+	-	+
Fructose	-	-	-	+	-	+
Maltose	-	-	-	+	-	+

Table 7. *Gram staining and microscopic results.*

Species	Shape	Colour	Gram staining
A	Rods	Pink	Negative
B	Rods	Pink	Negative
C	Rods	Pink	Negative
D	Spherical	Pink	Positive
E	Rods	Faint Reddish	Negative
F	Spherical	Purple	Positive

Biochemical tests

The six bacterial species isolated from the surface samples were identified using Biochemical tests and the results obtained are shown in Table 6.

Gram staining

Six bacteria species isolated from surface samples were analyzed using the gram staining method and results are summarized Table 7.

Bacteria confirmation using 16S PCR

The targeted universal conserved regions of the 16S gene were used to generate mixed community PCR products used to develop clone libraries. Figure 3 shows the electrophoresis gel of the 16S PCR products against a 100 bp ladder.

Bacterial identification

From the biochemical tests selective media and 16S PCR that were done, the six bacterial species were then identified as follows:

A *Escherichia coli*
B *Shigella*
C *Salmonella*
D *Staphylococcus*
E *Pseudomonas*
F *Streptococcus*

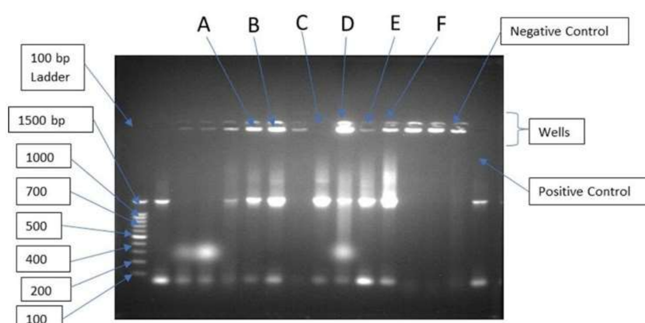

Figure 3. *Electrophoresis gel after 16S ribosomal DNA amplified products against a 100 bp ladder.*

Discussion

Proper water management and good hospital hygiene are essential in preventing HAIs, which can be particularly harmful to patients with weakened immune systems (Cissé et al., 2023).

Table 3 shows various water physicochemical parameters for three hospitals (A, B, and C), along with the overall statistics for all hospitals combined. The parameters included temperature, pH, total dissolved solids (TDS), and residual chlorine. The results were then compared to the Zimbabwean

Standard (SAZ) for water quality. The temperature ranges in all three hospitals were within acceptable limits. Temperature alone is not a direct indicator of the presence or absence of pathogenic bacteria, but extremes in temperature can impact water quality (Nijhawan & Howard, 2022; Richiardi et al., 2023). The pH levels in all three hospitals were within the SAZ guidelines. Maintaining a pH within the specified range is crucial for preventing corrosion of pipes and ensuring the effectiveness of disinfection processes. TDS measures the concentration of dissolved substances in water. The TDS values for all hospitals were within the acceptable range. Elevated TDS levels could indicate contamination, but in this case, they were within the Zimbabwean standards. Residual chlorine is often used as a disinfectant in water treatment to control pathogenic bacteria. The residual chlorine levels for all hospitals were within the specified range, suggesting that there is an effective disinfection process in place among all three hospitals. The fact that all the hospitals are monitoring and reporting these physicochemical parameters suggests a commitment to water quality management. Regular monitoring and maintenance help ensure that any deviations from standards are promptly addressed. The implementation of guidelines from organizations like the World Health Organization (WHO) and local health authorities could also play a role in maintaining water quality. Microbial testing, including tests for coliform bacteria were also done in this study and results were negative for all water samples collected from all three hospitals.

Surfaces in the ICU can serve as reservoirs for nosocomial pathogens, contributing to disease spread (Russotto et al., 2015). Recent studies highlight the persistence of pathogens on surfaces and emphasize the importance of rigorous environmental cleaning to mitigate transmission risks (Wißmann et al., 2021). The prevalence of bacteria obtained on surfaces of the 3 hospitals proved to vary across the different healthcare settings, emphasizing the need for tailored infection control measures in the ICUs. However, a number of factors were identified, contributing to higher bacterial load in ICU surfaces of hospital A. Hospital A lacked proper ICU standards, being characterized by broken windows, continuous use of gloves over a long period by the staff, storing of water in a container for long period of time. All three ICU environments lacked temperature control units, and this led to the creation of conditions conducive to bacterial growth.

All three facilities showed the presence of *E. coli* on surfaces as *E. coli* is a common commensal bacterium in the human gastrointestinal tract (Martinson & Walk, 2020), and all three ICU wards admit individuals who are under critical care and cannot visit the toilet on their own. These individuals end up messing on themselves and they end up increasing the bacterial load. *Staphylococcus* was also found to be prevalent in all three healthcare facilities, this might be due to its ability to colonize the skin and mucous membranes. Surgical procedures, invasive devices, and compromised skin integrity increase the risk of *Staphylococcus* infections (Seidelman et al., 2023). *Pseudomonas* was also found to be prevalent in all three hospitals, especially on the patient monitor and the patient handbook, this was because *Pseudomonas* is an opportunistic pathogen often associated with healthcare settings (Naily et al., 2023). Its presence in the ICU may be linked to the movement of objects from other wards, contaminated medical equipment, and failing to sterilize everything that enters the ICU ward. *Streptococcus* was also found to be prevalent in all three hospitals. The microorganisms were found to be mostly prevalent on the trays, tables, ventilators, and the bedside locker, this might be due to that, *Streptococcus* are common inhabitants of the human respiratory tract and mucous membranes (Okeke et al., 2005).

Hospital B tested positive for *Shigella* species. *Shigella* is typically associated with fecal-oral transmission. Poor hand hygiene, contaminated medical equipment, or exposure to infected patients may have contributed to its presence in the ICU of Hospital B, especially in cases of diarrheal illnesses. Similar to *Shigella, Salmonella* is often associated with foodborne or waterborne transmission (Kim, 2010). However the presence of the *Salmonella* was identified from samples that were collected from Hospital A, and these were from swabs that sampled the floor of the facility.

Conclusions

A significant amount of potentially harmful pathogenic bacteria on the surfaces of the ICUs suggests a higher chance of these pathogens entering and spreading throughout the facilities. However, the water quality was found to be within the Zimbabwean drinking water standards (SAZ), and negative for any bacteria in all the 3 ICUs. Therefore, there is a need to continue checking on the quality of water, and implementation of guidelines from the World Health Organization (WHO) on proper ICU standards.

Acknowledgement

The Department of Biotechnology and Biochemistry is acknowledged for providing reagents used in this study.

References

Bardossy AC, Zervos J, Zervos M, 2016. Preventing hospital-acquired infections in low-income and middle-income countries: impact, gaps, and opportunities. Infect. Dis. Clin. North Am., 30: 805-818.

Bitton G, 2014. Microbiology of drinking water production and distribution. John Wiley & Sons, Inc., p. 91-115.

Cissé D, Laure E, Blaise K., Paul N, Gbonon M, Mayaka C, Eugénie G, Simplice D., Philippe K., Mamadou S, 2023. Evaluation of the implementation of hospital hygiene components in 30 health-care facilities in the autonomous district of Abidjan (Cote d'Ivoire) with the WHO Infection Prevention and Control Assessment Framework (IPCAF), BMC Health Services Research, 23: 1-11.

Harun G, Anwar M, Sumon S, Hassan Z, Haque T, Muneer S, Rahman A, Abdullah S, Islam S, Styczynski A, Kaydos-Daniels S, 2022. Infection prevention and control in tertiary care hospitals of Bangladesh: results from WHO infection prevention and control assessment framework (IPCAF), Antimicrobial Resistance & Infection Control, 11: 1-13.

Kim S, 2010. Salmonella serovars from foodborne and waterborne diseases in Korea, 1998-2007: total isolates decreasing versus rare serovars emerging. J Korean Med. Sci. 25: 1693–1699.

Martinson J, Walk S, 2020. *Escherichia coli* residency in the gut of healthy human adults. EcoSal Plus., 9: 1-27.

Mathur P, 2018. Prevention of healthcare-associated infections in low- and middle-income countries: The 'bundle approach', Indian J Med Microbiol. 36: 155-162.

Naily W, Sunardi S, Asdak C, Dida E, Hendarmawan H, 2023. Distribution of *Escherichia coli* and coliform in groundwater at Leuwigajah and Pasirkoja Areas, West Java, Indonesia. Earth Environ. Sci., 1201: 1-8.

Nijhawan A, Howard G, 2022. Associations between climate variables and water quality in low- and middle-income countries: A scoping review. Water Res., 210: 1-11.

Noor A, Ishaq A, Jafri L, Jabeen F, Rani R, Kiani B, Akhtar N, Javed Z, Younis Jalal T., 2021. Health Care Associated Infections (HCAIs) a new threat for world; U-Turn from recovery to death, Campylobac., doi: 10.5772/intechopen.97193.

Okeke I, Klugman K, Bhutta Z, Duse A, Jenkins P, O'Brien T, Pablos-Mendez A, Laxminarayan R, 2005. Antimicrobial resistance in developing countries. Part II: strategies for containment. Lancet Infect. Dis., 5 :568-80.

Richiardi L, Pignata C, Fea E, Bonetta S, 2023. Are indicator microorganisms predictive of pathogens in water? Water, 15: 1-30.

Russotto V, Cortegiani A, Raineri S, Giarratano A, 2015. Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. J Intensive Care, 3: 1-8.

Seidelman J, Mantyh C, Anderson D, 2023. Surgical site infection prevention: a review. JAMA, 329: 244-252.

Wißmann J, Kirchhoff L, Brüggemann T, Steinmann D, Steinmann E, 2021. Persistence of pathogens on inanimate surfaces: a narrative review. Microorganisms. 9: 1-36.