ISSN 2413-0516

Prevalence and Bacteriological Profile of Healthcare-Associated Pathogens Isolated from Basra Hospitals

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(Submitted: 13 October 2024 – Revised version received: 26 October 2024 – Accepted: 15 November 2024 – Published online: 26 December 2024)

Abstract

Objective: Nosocomial infections-especially bacterial infections- are a major concern in healthcare, creating significant risks to both patients and healthcare staff. Given the prevalence of nosocomial infections, the lack of this type of study in Basra province, southern Iraq, is a significant oversight that must be addressed. For the above reason, this study amid to provide appropriate diagnosis for pathogenic bacteria isolated from intensive care unit (ICUs) and operating room (OR) environments at three major hospitals in Basra southern Iraq and to identify the source of bacterial contamination inside these hospitals.

Methods: Two hundred and ten swab samples were collected from ICU and OR environments including inanimate objects, medical devise, health care attire. Bacteria were isolated using standard microbiology techniques. VITEK® 2 system and 16S rDNA sequencing were used for bacterial identification.

Results: Showed 69 (32.857%) samples were positive for bacterial growth. 39 (56.521%) isolates classified as Gram-positive, and 30 (43.478%) isolates classified as Gram-negative. According to the VITEK®2 system and 16S rDNA sequencing, the most prevalent species among Gram positive bacteria was *Staphylococcus aureus* with 10 (14.493%) isolates. While the most prevalent species among Gram negative was *Klebsiella pneumoniae* with 8 (11.594%) isolates.

Conclusions: The study revealed a moderate degree of bacterial contamination on surfaces and equipment within ICUs and ORs of the investigated hospitals.

Keywords: Bacterial Infections, hospital acquired infections (HAIs), intensive care units (ICUs), nosocomial infections

Introduction

Nosocomial infections or hospital acquired infections (HAIs) are a major concern in healthcare, creating significant risks to both patients and healthcare staff. Two specifically vital areas affected by these infections: Intensive Care Units (ICUs) and Operating Rooms (ORs). Growing evidence suggests that contaminated hospital surfaces contribute significantly to the spread of healthcare-associated infections, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Clostridium difficile*, and norovirus.[1]

Outbreaks have linked to the bacterial colonization of inanimate surfaces in ICU[2,3] and cross-transmission of disease-causing bacteria between patients.[4,5] Bacterial pathogens are the most frequent pathogens due to their potential resistance for disinfection,[6] capability of surviving for extended periods on inanimate, desiccated surfaces, with greater longevity in moist, colder conditions.[7] Factors that influence bacterial contaminations are: primary factors (microorganisms type, source of infection, destination surfaces, level of humidity, and inoculum size)[8,9] and secondary factors (adherence to handwashing practices, Nurse-to-patient ratios, infections prevalence, ICUs layout (single-patient or multiple-patient rooms), usage of antibiotic prescribing guidelines).[10]

Moreover, room occupancy history is a significant risk factor for acquiring infections like MRSA, VRE, *Clostridium difficile*, and multidrug-resistant organisms. Despite recommendations for routine and terminal disinfection, inadequate cleaning practices remain a major concern.[1]

The absence of such research in Basra province, southern Iraq, underscores the need for its implementation to mitigate nosocomial infections. Therefore, this study was amid to provide appropriate characterization for pathogenic bacteria in ICUs and ORs at three major hospitals in Basra and to detect the source of bacterial contamination inside these hospitals.

Materials and Methods

Study Design and Period

A hospital based cross-sectional study was performed at ICU and OR environments of Al-Basra Teaching Hospital, Al-Fayhaa Teaching hospital and Al-Mawani Teaching hospital from January 2, 2024 to May 30, 2024.

Sampling

Two hundred and ten duplicate swab (Global Scientific, UK) samples (wet and dry) were collected from ICU and OR at morning, taking into account the most representative hours at 8:00 AM–2:00 PM. The frequently touched areas were swabbed in a close zigzag pattern at each site, turning the swab during sample collection to ensure that the complete area of the swab is used. the sites of sampling were classified into: (1) Medical instruments including face mask, anaesthesia machine, suction device, laryngoscope, monitor device, screen display of the X ray machine, oximeter, and oxygen cylinder. (2) inanimate surfaces including patient beds surfaces, wall surfaces, door handle, tables, chairs, cabinets, sinks, gowns of medical staffs, bed clothes and patient chart. (3) Hands of healthcare providers (fingers and palm area of the hands as well as mobile phone).[6]

Bacterial Isolation

Following sample collection, all collected swabs were transported to the microbiology laboratory within 30 minutes to

one hour, instantly cultured on blood agar (Himedia, USA) and MacConkey agar (Himedia, USA) by streaking method and incubated in aerobic conditions at 37°C for 24–48h and observed for any bacterial growth.

Bacterial Identification

Identification of bacteria was done using standard microbiological techniques. The characteristics of grown colonies have been identified phenotypically by culturing on selective media (Eosin Methylene Blue agar, MacConkey agar, *Shigella Salmonella* agar and Mannitol Salt agar (Himedia, USA)) and Gram staining. Identification with the VITEK® 2 system was achieved using ID-Gram Negative (ID-GN) cards and ID-Gram Positive (ID-GP) cards, according to the manufacturer's instructions. (bioMérieux, France). The 64 well ID-GN and ID-GP cards contain 43–47 tests measuring carbon source utilization, enzymatic activities, and resistance. A vacuum device is used to inoculate the cards with a suspension of the organism equal to 0.5 McFarland standard prepared from 18–20 h old culture. The cards are sealed automatically and inserted manually into the VITEK®2 system. Fluorescence readings are obtained every 15 minutes, and final results were available in approximately 8–10 hours. VITEK® 2 analysis was done at the scientific source company for training and development, Bagdad, Iraq.

16S rDNA Gene Amplification and Sequences

amplifying the 16S rDNA was done using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The reaction mixtures contained 1.5 µl DNA template, 1.5 µl forward primer (10 pmol), 1.5 µl reverse primer (10 pmol), 11 µl AccuPower® PCR PreMix (Bioneer, Korea) and 34.5 µl nuclease-free water in a final volume of 50 µl. The amplification protocol were as follows: initial denaturation at 92°C for 2 min followed by 30 cycles each consisted of denaturation at 94°C for 30s, annealing at 51.8°C for 45 s and extension at 72°C for 1 min and 30s, with a final extension at 72°C for 5 min. PCR product was separated on a 2% agarose gel with 1% ethidium bromide, the 16S rDNA bands were visualized under UV illuminator and photographed.[11] Genomic DNA extraction and amplification were done at the scientific source company for training and development, Bagdad, Iraq. PCR products were sent to MACROGEN Co./Korea for sequencing.

Data Analysis

Descriptive analysis was used to define the samples and compare the results. All data were analysed using JASP statistical software.

Results

Two hundred and ten samples were enrolled in this study, 69 (32.857%) of which were positive for bacterial growth which was collected from different sites in ICUs and OR as appeared in Tables 1 and 2.

Analysis of contamination within the ICU and OR environments revealed that suction devices were the most frequently contaminated. Patient's bed and bed clothes followed. Conversely, the lowest contamination levels were observed on oximeter. Tables 3, 4 and 5 details the distribution of these cultures by location and species type.

The Gram stain classified the pure cultures into: 39 (56.521%) isolates identified as Gram-positive, and 30 (43.478%) isolates identified as Gram-negative. According to the VITEK®2 system and DNA sequence results, the most prevalent species among Gram positive bacteria was *Staphylococcus aureus* with 10 (14.493%) isolates found, followed by *Enterococcus faecalis* with

Table 1. **The primary and secondary sites of swabbing in OR**

Primary sites	Secondary sites
Otorhinolaryngology unit	Mask of O_2 supply, Anaesthesia machine, Suction device, Laryngoscope Patient beds, Walls, Door handle, Floors
Urology Unit	Mask of O_2 supply, Anaesthesia machine, Suction device, Laryngoscope Patient beds, Walls, Door handle Cabinets, Sinks
Ophthalmology Unit	Mask of O_2 supply, Anaesthesia machine Suction device, Laryngoscope, Patients beds, Walls
Orthopaedics Unit	Mask of O_2 supply, Anaesthesia machine Suction devices, Laryngoscope, Monitor device, Walls, Cabinets, Sinks, Hands of health care providers (fingers) Hands of health care providers (plam area of the hands), Health care provider's phone
Obstetrics and gynaecology unit (Gynae & Obst)	Mask of O_2 supply, Anaesthesia machine Suction device, Laryngoscope, Patient beds, Walls, Sinks
Surgical unit	Mask of O_2 supply, Anaesthesia machine, Suction device, Laryngoscope, Monitor device, Oximeter, Patients beds, Walls Cabinets
Neurosurgery unit	Mask of O_2 supply, Anaesthesia machine Suction device, Patients beds, Walls, Floors
Total	122 swabs

Table 2. **The primary and secondary sites of swabbing in ICUs***

Primary sites	Secondary sites
ICU-1	Mask of O_2 supply, Walls, Monitor devices Patient beds, Suction device
ICU-2	Mask of O_2 supply, Suction device, Monitor device, Patients beds, Walls, Patient charts
ICU-3	Mask of O_2 supply, Suction device, Walls Patient beds
ICU-4	Monitor device, Patients beds, Walls, Patient charts, Floors
ICU-5	Suction device, Mask of O_2 supply, Monitor device, Patients beds
ICU-6	Monitor devices, Patients beds, Patient charts, Floors
Total	88 swabs

* Two ICUs from each hospital.

Table 3. **Number and percentage of the total samples and culture-positive samples isolated from OR**

Site	Total culture	Percentage %	No of positive culture	Percentage %
Otorhinolaryngology	8	6.56	2	4.082
Urology	15	12.30	5	10.2
Ophthalmology	15	12.30	2	4.082
Orthopaedics	31	25.41	11	28.57
Gynae & Obst	15	12.30	8	16.33
Surgical	30	24.59	16	32.65
Neurosurgery	8	6.56	2	4.082
Total	122	58.10	46	23.33

Table 4. **Number and percentage of the total samples and culture-positive samples isolated from ICUs**

Site	Total culture	Percentage %	No of positive culture	Percentage %
ICU-1	12	13.6	2	8.70
ICU-2	14	15.9	2	8.70
ICU-3	16	18.2	7	30.43
ICU-4	15	17	2	8.70
ICU-5	17	19.3	5	21.74
ICU-6	14	15.9	5	21.74
Total	88	41.9	23	26.14

Table 5. **Number and distribution of bacterial isolates on the sites of swabbing**

Bacterial species / **Sources**	***Burkholderia cepacia***	***Klebsiella pneumoniae***	***Enterococcus faecalis***	***Staphylococcus aureus***	***Staphylococcus haemolyticus***	***Streptococcus parasanguinis***	***Kocuria kristinae***	***Enterobacter cloacae***	***Acinetobacter lwoffii***	***Pseudomonas stutzeri***	***Escherichia coli***	***Enterococcus casseliflavus***	***Staphylococcus epidrmidis***	***Staphylococcus warneri***	***Staphylococcus hominis***
Mask of O_2 supply	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
anesthesia machine	+	+	+		-	-	-	-	-	-	-	-	-	-	-
suction device	+	+	+	+	+	+	-	+	-	-	+	-	-	-	-
laryngoscope	-	+	+	+		+	-	+	-	-		-	-	-	-
monitor device	-		+		+	-	-	+	-	-		-	-	-	-
screen display of the X ray machine	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
oximeter	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Patient's beds		-	+	+	-	-	-	-	-	+	+	-	-	-	-
Walls & floors	-	-	+		-	-	-	-	-	+		+	-	-	-
door handle	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+
tables	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
chairs	-	-	-		-	-	-	-	+	-	-	+	-	-	-
cabinets	-	-	-		-	-	-	-	+	-	-	-	-	-	-
sinks	-	-	+		-	-	-	-	-	+	+	-	-	-	-
gowns of medical staffs	-	+	+	+	-	-	-	-	+	-	+	+	-	+	-
patient chart	-	-	-	+		+	+	-	-	-	+		+	-	-
Hands of healthcare providers (fingers)	-	+	-	+	-	+	+	+	+	-	-	+	+	-	-
Hands of healthcare providers (palm area of the hands)	-	+	-	+	-	-	+	+	+	-	-	-	-	-	-
healthcare provider's phone	-	+	-	+	+		+	+	+	-	-	-	-	+	-

Table 6. **Number, percentage and type of bacteria identified in the ICUs and ORs**

Bacteria	No of sample	Percentage %	Bacterial type
Burkholderia cepacia	4	5.797	-Ve
Klebsiella pneumoniae	8	11.594	-Ve
Enterococcus faecalis	8	11.594	+Ve
Staphylococcus aureus	10	14.493	+Ve
Staphylococcus haemolyticus	4	5.797	+Ve
Staphylococcus warneri	2	2.899	+Ve
Staphylococcus epidrmides	2	2.899	+Ve
Staphylococcus hominis	1	1.449	+Ve
Streptococcus parasanguinis	4	5.797	+Ve
Kocuria kristinae	4	5.797	+Ve
Enterobacter cloacae	6	8.696	-Ve
Acinetobacter lwoffii	4	5.797	-Ve
Pseudomonas stutzeri	2	2.899	-Ve
Escherichia coli	6	8.696	-Ve
Enterococcus casseliflavus	4	5.797	+Ve
Total	69	100%	----

Table 7. **Isolates identification by 16S rDNA sequencing**

No	Bacterial species	Similarity	Accession number	Base pair length
1	*Burkholderia cepacia*	100%	U96927.1	1050bp
2	*Burkholderia cepacia*	100%	MW534387.1	961bp
3	*Burkholderia cepacia*	100%	AB334766.1	1155bp
4	*Burkholderia cepacia*	100%	PP670005.1	997bp
5	*Klebsiella pneumoniae*	100%	OM045059.1	361bp
6	*Klebsiella pneumoniae*	100%	PP504940.1	1117bp
7	*Klebsiella pneumoniae*	100%	PP497003.1	567bp
8	*Klebsiella pneumoniae*	100%	OP001794.1	1187bp
9	*Klebsiella pneumoniae*	100%	PQ114161.1	1023bp
10	*Klebsiella pneumoniae*	100%	KU936064.1	1121bp
11	*Klebsiella pneumoniae*	100%	OQ128082.1	368bp
12	*Klebsiella pneumoniae*	100%	PP515608.1	759bp
13	*Enterococcus faecalis*	100%	NR_040789.1	1036bp
14	*Enterococcus faecalis*	100%	OR016180.1	1114bp
15	*Enterococcus faecalis*	100%	OQ644518.1	1036bp
16	*Enterococcus faecalis*	100%	MK571202.1	513bp
17	*Enterococcus faecalis*	100%	MW320714.1	370bp
18	*Enterococcus faecalis*	99%	MN326674.1	707bp
19	*Enterococcus faecalis*	99%	LR991660.1	744bp
20	*Enterococcus faecalis*	99%	KP662075.1	783bp
21	*Staphylococcus aureus*	100%	OP889689.1	1131bp
22	*Staphylococcus aureus*	100%	MT280152.1	760bp
23	*Staphylococcus aureus*	100%	PP291860.1	1057bp
24	*Staphylococcus aureus*	100%	PP197164.1	814bp
25	*Staphylococcus aureus*	100%	OM936855.1	685bp
26	*Staphylococcus aureus*	100%	OQ581797.1	559bp
27	*Staphylococcus aureus*	100%	MT416445.1	793bp
28	*Staphylococcus aureus*	99%	MN606179.1	1239bp
29	*Staphylococcus aureus*	99%	HM307769.1	796bp
30	*Staphylococcus aureus*	99%	OR462684.1	1062bp
31	*Staphylococcus haemolyticus*	100%	NR_036955.1	905bp
32	*Staphylococcus haemolyticus*	99%	MZ636452.1	1176bp
33	*Staphylococcus haemolyticus*	100%	KF092983.1	1183bp
34	*Staphylococcus haemolyticus*	100%	GQ079095.1	748bp
35	*Staphylococcus warneri*	100%	MZ768708.1	1199bp
36	*Staphylococcus warneri*	100%	OK090515.1	1104bp
37	*Staphylococcus epidrmides*	100%	MG027640.1	633bp
38	*Staphylococcus epidrmides*	100%	MT573042.1	590bp
39	*Staphylococcus hominis*	100%	NR_036956.1	1201bp
40	*Streptococcus parasanguinis*	100%	NR_024842.1	1199bp
41	*Streptococcus parasanguinis*	99%	AF543299.1	150bp
42	*Streptococcus parasanguinis*	99%	HM596296.1	741bp
43	*Streptococcus parasanguinis*	99%	NR_115241.1	596bp
44	*Kocuria kristinae*	100%	JX861555.1	1066bp
45	*Kocuria kristinae*	99%	KC581674.1	1087bp
46	*Kocuria kristinae*	99%	KR230389.1	1040bp
47	*Kocuria kristinae*	99%	FR682682.1	490bp
48	*Enterobacter cloacae*	100%	LT221670.1	1448bp
49	*Enterobacter cloacae*	100%	MT557028.1	801bp
50	*Enterobacter cloacae*	100%	KF516281.1	604bp
51	*Enterobacter cloacae*	99%	OR426303.1	952bp
52	*Enterobacter cloacae*	99%	OQ171571.1	521bp
53	*Enterobacter cloacae*	99%	MN173459.1	463bp
54	*Acinetobacter lwoffii*	100%	KC816553.1	1459bp
55	*Acinetobacter lwoffii*	100%	KC178575.1	868bp
56	Acinetobacter lwoffii	100%	KF737156.1	620bp
57	Acinetobacter lwoffii	100%	LN774431.1	766bp
58	*Pseudomonas stutzeri*	100%	MK007478.1	980bp

Table 7. **Isolates identification by 16S rDNA sequencing—*Continued***

59	*Pseudomonas stutzeri*	100%	PP780384.1	705bp
60	*Escherichia coli*	100%	OM881897.1	1090bp
61	*Escherichia coli*	100%	MT320156.1	1020bp
62	*Escherichia coli*	100%	OM882311.1	810bp
63	*Escherichia coli*	100%	ON921243.1	755bp
64	*Escherichia coli*	100%	OQ171488.1	570bp
65	*Escherichia coli*	100%	MK621249.1	1211bp
66	*Enterococcus casseliflavus*	100%	MF959774.1	840bp
67	*Enterococcus casseliflavus*	100%	LT745978.1	466bp
68	*Enterococcus casseliflavus*	100%	KJ571214.1	590bp
69	*Enterococcus casseliflavus*	99%	KM096606.1	970bp

8 (11.594%) isolates. While, the most prevalent species among Gram negative was *Klebsiella pneumoniae* (8 (11.594%) isolates). Followed by 6 (8.696%) isolates of both *Escherichia coli* and *Enterobacter cloacae* as mentioned in Tables 6 and 7, Figures 1 and 2.

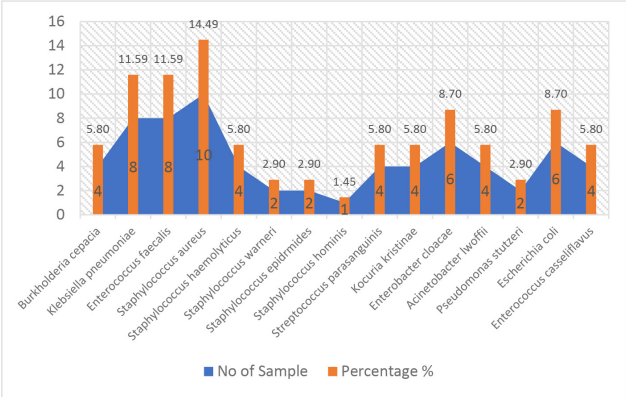

Fig. 1 **Percentage of microbes identified in the ICUs and OR of the hospitals.**

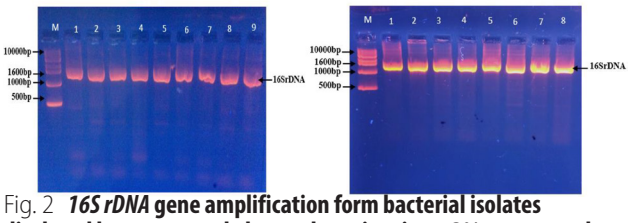

Fig. 2 ***16S rDNA* gene amplification form bacterial isolates displayed by agarose gel electrophoresis using a 2% agarose gel containing ethidium bromide: Lane M: 1kb DNA ladder, Lane 1-9: *16S rDNA* gene bands of bacterial isolates.**

Discussion

Nosocomial infections impose a huge economic burden on hospitals, for example, pneumonia and bloodstream infections, can triple the fatality risk in immunocompetent patients. Accordingly, a significant percentage of patients in the ICUs dies because of their hospital-acquired infections, rather than their underlying illness.[12] Results of this study indicated a moderate level of bacterial colonization in the OR (23.33%) and ICU (8.571%) areas in the investigated hospitals. Surprisingly, the level of bacterial contamination in OR appeared in this study was noticeably lower as compared to findings of Baban et al. and Al-Juboory &c which showed higher contamination (35.6%) and (41.01%) in one of the Erbil and Musel hospitals respectively.[12,13] Despite the low levels of bacterial contamination in ORs, there is still contamination, which is attributed to inadequate obedience to infection control procedures in the studied hospitals.

ICU patients are in contact with various monitoring devices and life support equipment. Several studies, observational and infection control reports, suggested the non-invasive ICU instruments role as a possible cause of nosocomial infections and in the most cases, contamination has involved electrical instruments or hard to clean equipment due to hidden surface areas or inadequate disinfection.[14] In this study, medical instruments like tubing of sucker machine, sucker tip, laryngoscope and monitor devices demonstrated a high level of bacterial growth which were in consistent with Javed et al. and Sui et al.[15,16]

Furthermore, mobile phone and patient charts also exhibited a bacterial growth. Medical charts are susceptible to bacterial colonization of their surface since they are frequently used by healthcare attire including doctors, and nurses for recording case notes after patient contact for physical assessments or invasive protocols. Medical charts transportation between wards increases the risk of surface colonization. Several reports examined the contamination of medical charts outer surfaces in ICUs and shown that charts can have a high rate of contamination, reaching 80–90%.[17,18]

Meanwhile, mobile phones are the most widely utilized non-medical portable electronic instruments in ICUs. They are not only utilized for interacting but also for online consultation and applications use for patient's care. Many studies have emphasized the severe mobile phones colonization by bacteria, including multi drug resistant bacteria (MDR).[19]

Based on the results in this study, Gram positive cocci *Staphylococcus aureus* and *Enterococcus faecalis* and Gram-negative enterobacteria *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli* were the most common isolates. Al juboory & Abdul Aziz recorded *Staphylococcus aureus* was the highest isolation with 17.70% of the total isolates.[13] Baban et al. and Ayçiçek et al. also reported the highest rate of contaminant bacteria was for *Staphylococcus aureus* with (70%) and (78.8%) respectively.[12,20] The source of contamination was detected from the healthcare attire, or from the skin flora of the patient. Staphylococcal bacteria, both coagulase negative and coagulase positive exhibit an enthusiastic ability to survive in various environmental conditions including wide range of temperatures, humidity levels, exposure to sunlight and resistance to desiccation. Results of DNA typing procedure from outbreaks in hospitals shown persistence of Staphylococcal bacteria up to 5 years.[9]

Moreover, Ekrami et al. found that Gram-negative enterobacteria (*Klebsiella pneumoniae*, and *Enterobacter* spp.) were

the predominant isolates among Gram negative bacteria.[21] Gram negative bacteria are often known as the main cause of hospital acquired infections with *Klebsiella spp* as the primary pathogens isolated.[22] The presence of coliform or enterobacteria on the hands is an indicator for fecal oral contamination and also poor hand hygiene.

Conclusions

The study revealed a moderate degree of bacterial contamination on surfaces and equipment within ICUs and ORs of the investigated hospitals. Among, the isolated bacteria, Gram-positive appeared slightly more frequent than Gram-negative bacteria. *Staphylococcus aureus* was the most predominant species of Gram-positive bacteria, while *Klebsiella pneumoniae* was the most predominant species of Gram-negative bacteria.

Acknowledgment

Authors extend their sincere gratitude to the management and staff of Basra Teaching Hospital, Al-Fayhaa Teaching Hospital, and Al-Mawani Teaching Hospital for their assistance in sampling.

Conflicts of Interest Disclosure

The Authors declare no conflicts of interest.

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