Gaza AMR Pilot Study

Anti-Microbial Resistant Bacteria in Health Care Facilities: exploring links with WASH

Final Report

Reem Shomar, February, 2021

1. Abstract

Background: Antimicrobial resistance (AMR) is a growing global phenomenon that refers to microorganisms' ability to survive exposure to an antimicrobial agent to which it was previously sensitive.

Rationale: The link between WASH services and AMR risk remains unclear, in particular at healthcare settings in countries with deteriorated WASH services.

Methods: A hospital-based cross-sectional study to detect and identify antimicrobial resistance bacteria was conducted. Random samples from water, wastewater, soap, and surface swabs (n=345) were collected from Al-Shifa and European Gaza hospitals and screened for the presence *Enterobacteriaceae*, *Pseudomonas*, *Enterococcus* and *Staphylococcus aureus*. Antimicrobial susceptibility, ESBL production, Carbapenem resistance, and AMR genes were investigated.

Results: High levels of bacterial contamination was detected in water and surface swab samples with an overall percentage 34.1%. Of the total positive microbial growth, (35.7%) on m-Endo Agar, (25.2%) on Manitol Salt Agar, (23.8%) on Cetrimide Agar; and (12.2%) on m-Enterococcus Agar. Twenty-two percent of identified Enterobacteriaceae was positive for ESBL and fourteen percent was positive for Modified Hodge test (MHT). Over 2/3 of isolated Enterobacteriaceae in water and wastewater samples found resistant to Amikacin, Ceftazidime, Ceftriaxone, and Imipenem. All *Enterobacteriaceae* isolates from swab samples were found to be resistant to Pipracillin-Tazopactam, Amikacin, Ceftazidime, and Ceftriaxone. The prevalence of ESBL genes among *Enterobacteriaceae* isolates were: 11.1% for NDM, 25% OXA, 19.4% SHV, 2.8% KPC, 66.7% TEM, 41.7% blaCTXM, and 5.6% blaCTXM-3. For Carbapenem resistant gene (MDM), the prevalence among *Enterobacteriaceae* was 11.1% and among *Pseudomonas* was 12.5%. The antibiotic susceptibility profile was also presented for *Pseudomonas*, *Enterococcus* and *S. aureus*.

Conclusion: The results of this study underlined the level of contamination with AMR bacteria in samples collected from WASH services at healthcare settings and highlighted the need to consider the safety of WASH service provided at health care facilities as an essential aspect in the fight against the spread of AMR.

2. Introduction

Proper Water, Sanitation and Hygiene (WASH) services in health care settings are essential for the quality of provided healthcare and for the safety of patients, health workers, and served communities. Antimicrobial resistance (AMR) is the ability of a disease-causing organism to survive exposure to an antimicrobial agent to which it was previously sensitive. The phenomena renders traditional antimicrobials treatment (e.g. antibiotics) useless, unable to treat infections that can spread to others – particularly when inadequate sanitary conditions and poor infection control measures exist (Holmes et al., 2016). The concern with AMR is so dire that the Secretary-General of the UN issued a *Global Call to Action* in 2018 to provide improved WASH in all health care facilities (WHO & UNICEF, 2016).

According to the WHO-Unicef *Health Care Facilities Global Action Plan on Water Sanitation and Hygiene* (2016) investing in WASH at health care facilities would have multiple benefits such as enhancing the occupational health and safety, *and* reducing acquired infections and AMR (Mendelson & Matsoso, 2015)– see Figure 1. To date, however, there has been very little research that tests the links between WASH and AMR in health care facilities, and the world remains with an incomplete understanding the transmission routes or extent of damage that may be caused (Watson et al., 2019). This WASH-HCF-AMR study hypothesises that AMR pathogens can be transmitted at health care facilities through WASH services, and can reach the community through wastewater.

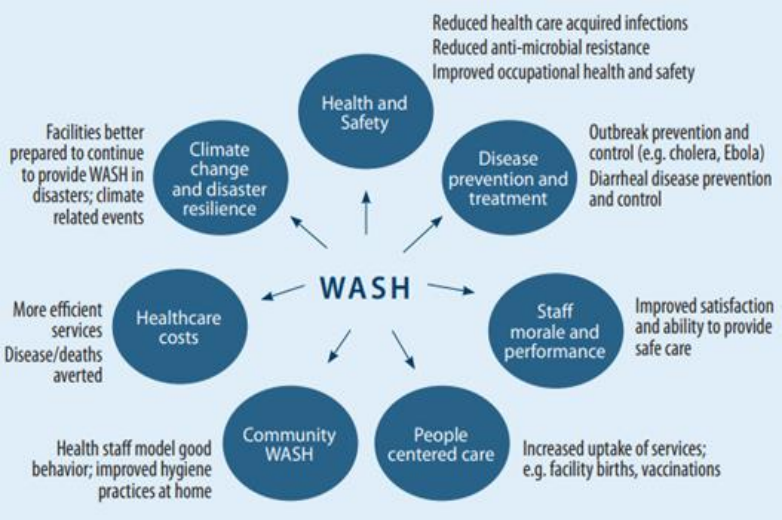

Figure 1: Benefits of investing in WASH in healthcare settings (WHO & UNICEF, 2016). Reduced anti-microbial resistance is just one of the benefits.

1.1. AMR in Gaza

There is a global concern about the increased incidence of resistance bacteria and their estimated death rates (about 700 thousand each year) which delay the progress against the third sustainable development goal "healthy lives and wellbeing for all" (O'Neill, 2016). The level of resistant bacteria in the Middle East is at alarmingly high levels particularly in conflict areas where the health systems, antimicrobial supplies and access to care are affected (Kanapathipillai et al., 2019). In Gaza this study isolated bacteria from clinical lab record samples including urine, stool, pus, sputum, blood, and other body fluids. The results of the microbiological survey in Gaza revealed that the most prevailing isolated bacteria in clinical samples were E. coli, *S. aureus*, K. pneumonia, P. aeruginosa and Enterococci, with clear evidence of microbial resistance to aminoglycosides, Penicillin's, b-lactams, and cephalosporin antimicrobials (Al Laham, 2012; Elmanama, Alyazji, & Abu-Gheneima, 2011; Hujier & Saleem, 2006; Tayh et al., 2017). This WASH-HCF-AMR Gaza pilot was designed in part to investigate whether such findings also held for its WASH-HCF focus.

1.2. WASH in Gaza

Gaza is the southern governorate of Palestine, a lower-middle income country, comprising a narrow strip of land along the Mediterranean Sea (Figure 2). There are roughly 1.9 million residents (representing about 40% of Palestinians living in the Palestinian territories) living over 365 km^2 area as of 2018 (PCBS, 2018a). Of them, only 11.4 % have access to safe water through networks (PCBS, 2018b).

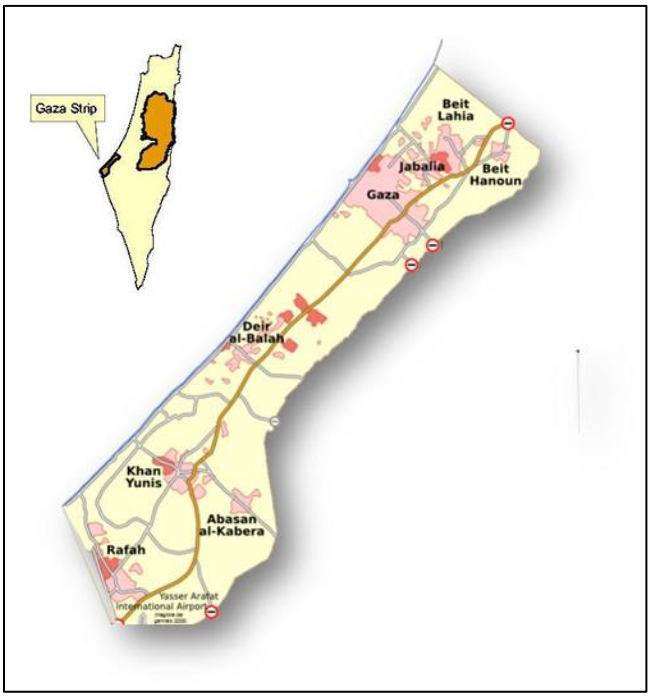

Figure2 : Gaza Strip Map

1.2.1. Water resources and drinking water quality in Gaza

The main source of water for all activities in Gaza is the very highly over-extracted shallow costal aquifer, which is under serious crises in terms of quality & quantity (PWA, 2018). The average daily per capita water consumption was 89 l/c/d while the minimum was recorded in southern Rafah Governorate (78.5 l/c/d), which is below the limit recommended by the WHO (100 l/c/d). The even greater water concern is with its quality, with 90% of municipal wells exceeding WHO drinking water guideline levels for nitrates and 79 % for chlorides – see Figure 3. Microbiological

contamination with total coliform was detected in about 22% of tested drinking water samples from desalinated sources, with 9% of them were positive for faecal coliform (PWA, 2019).

As a result, the bulk of drinking water supplied to homes does not meet WHO's minimum drinking water guidelines (for nitrates and chlorides, amongst others), and is therefore considered unfit for human use. The majority of Gazans purchase drinking water from unregulated private water vendors who depend on brackish water desalination treatment which is not overseen by the water authorities and expected to be unsafe (PWA, 2015).

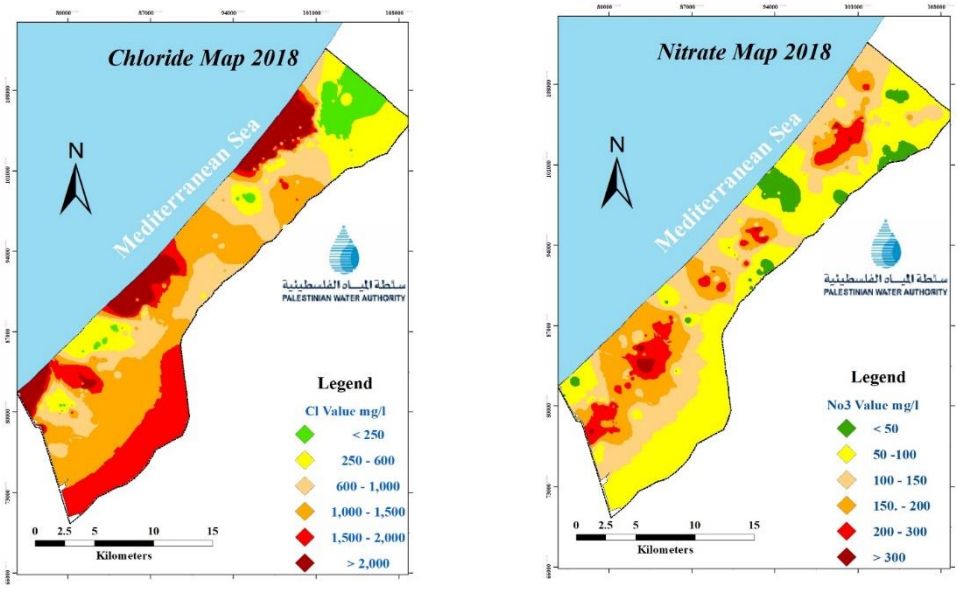

Figure 3 : Chloride and Nitrate Maps (PWA, 2018)

The water crisis has been exacerbated by the context of protracted conflict that all in Gaza live. Not dissimilar to Syria or Iraq, the context can be conceived of as an 'ecology' or 'biosphere' of war which multiplies the complexity and severity of AMR and WASH issues (Abu Sitta, Dewachi, Nguyen, & Whittall, 2016; Dewachi et al., 2014; Zeitoun & Abu Sitta, 2018). The existing situation hits the most vulnerable groups the hardest, particularly ill people, pregnant women, children, people with disability, and geographically marginalized people. Given that more than 33% are under extreme poverty line, the cost of purchasing drinking water from the water vendors increases the burden on those who are most vulnerable (World Bank, 2017). According to Social Determinants of Health theory, the impact of the long term deteriorated economic status

combined with a very low standard of living environment would be expected to affect the quality of life and therefore shape the people's health outcomes (Watts, Siddiqi, Shukrullah, Karim, & Serag, 2007; WHO, 2008).

1.2.2. Wastewater coverage and treatment in Gaza

Though roughly 78% of the Gazan population is connected to sewerage networks which lead to any of five operational wastewater treatment plants, inadequate wastewater treatment remains a grave environmental and health threat (World Bank, 2017). Due in part to the insecure and intermittent supply of the electricity, the efficiency of the treatment process is reduced, and the quality of the effluent (measured as Biological Oxygen Demand level) is typically beyond the maximum acceptable national standards for treated wastewater (CMWU, 2018). On a daily basis, more than 100 million liters of untreated or partially treated wastewater is discharged into the sea. Perhaps even more worryingly, some seeps through the sand cover into the underlying freshwater aquifer (OCHA, 2019; PWA, 2019; Shomar, Abdelatif, & Kishawi, 2017), thereby contaminating the main source of drinking water. health care facilities operate within this closed contaminated loop.

1.3. WASH in health care facilities in Gaza

The term 'Health care facilities' (HCF) refers to all formally recognized facilities that provide health care services (primary, secondary, and tertiary), including clinics and hospitals. All HCF involve private or public, permanent or temporary emergency structures. 'WASH in HCF' covers the provision of water, sanitation, health-care waste management, hygiene and environmental cleaning infrastructure, and services across all parts of the facility in question.

The key health care service providers in Gaza are the Ministry of Health (MoH) and United Nations Relief and Works Agency for Palestine Refugees in the Near East (UNRWA), as well as Non-Governmental Organizations (NGOs), and the private sector. Primary Health Care (PHC) is provided through 49 centers run by the MoH, 22 centers run by UNRWA and 70 centers run by the NGOs and the private sector. Secondary and tertiary health care is provided through 31 hospitals, of which the MoH operates fourteen hospitals (MoH, 2017).

According to the United Nations Office for the Coordination of Humanitarian Affairs (OCHA) humanitarian bulletin, Gaza's health care system is struggling to cope with the increased number of casualties and injuries – particularly after the Israeli response to the weekly Great March of Return demonstrations which began on 30 March 2018. The high number of injuries (over 4,000 in one day) add additional burden to a system already under considerable stress: the system suffers continuous shortage of essential drugs and medical consumables, insufficient resources to maintain the infrastructure, and intermittent and unreliable electricity.

Ensuring access to proper WASH services in healthcare facilities is part of the 2030 agenda for the global Sustainable Development Goals (SDGs), notably SDG 6. Related goals are SDG 3, which aims to ensure health and well-being for all at all ages, and SDG 9, which aims to reduce morbidities and mortalities from hazardous chemicals, water and soil contamination (Desa, 2016). The Joint Monitoring Programme (JMP) report issued by WHO and UNICEF found that basic water service was found around the world – remarkably - in only 25% of health facilities, thereby affecting 2 billion people. Meanwhile the absence of or inadequate sanitation service was found in 20% of health facilities, affecting 1.5 billion people. In addition, hand hygiene materials were missing in 16% of health care facilities (WHO, 2019; WHO & UNICEF, 2019). There has been little study but ample experience to suggest that the situation is similarly bad in Gaza.

1.3.1. Water supply in health care facilities

According to indicators of global WASH in HCF, the water supply must be available throughout the year from an 'improved' source located on premise. Improved water sources are those which have the potential to deliver safe water including: piped water, boreholes or tube wells, protected dug wells, protected springs, rainwater, and packaged or delivered water (WHO & UNICEF, 2019).

A recent assessment study by WeWorld-Gruppo di Volontariato Civile (WW-GVC) NGO for the availability "improved source accessed on premises" of 21 healthcare facilities in Gaza is telling. Over three-quarters (77%) were found to receive piped water from the municipality networks, while the remaining portion had onsite-protected wells, of which at least one well is observed to have high risk for contamination. Only 5% of the assessed HCFs has back up source for drinking

water (to be prepared for emergencies) and 48% of them had back up sources for domestic (e.g. washing) use. Irregular water quality monitoring is carried out by the designated unit with limited feedback response: only 43% of HCF were found to comply with standards. While water desalination units are available in 43% of the assessed HCFs, most of these were found to lack skilled staff and supplies needed for maintenance. Lack of cleaning and disinfection of water storage reservoirs is another source of contamination: about 6% of the assessed water storage reservoirs at the HCFs were found to be high risk, 57% have medium risk, and 37% have low risk of contamination. In addition, poor electrical supply has affected the availability of hot water in winter season and therefore affecting the hygiene and environmental cleaning practices (UNICEF & WW-GVC, 2019).

1.3.2. Sanitation in health care facilities

The guidelines for minimal sanitation services, an HCF should provide is usable and improved sanitation facilities, one sex-separated toilet with menstrual hygiene facilities, one toilet dedicated for staff, and one toilet for people with disability (WHO & UNICEF, 2019). This is to maintain a quality for care services that can improve the health outcomes for patients. Improper sanitation services will affect the health seeking behaviour and will reduce the workers' satisfaction. Immunocompromised patients, people with disability and children will be more likely to acquire health care associated infections including infections with antimicrobial resistance bacteria (Bouzid, Cumming, & Hunter, 2018).

In Gaza, only 19% of the previously assessed HCFs had basic sanitation services. None of the assessed facilities had children-adapted toilets and 76% of them had no accessible toilets to accommodate people with disability. In addition, in about half of the facilities, the same toilet is used by patients and working staff. The majority (n=20) of the assessed HCFs had their wastewater system connected to the municipal drainage networks without having wastewater pre-treatment units; this would suggest a high risk of discharging infectious and toxic medical wastewater to the public networks. Only one HCF had a wastewater treatment unit that performs primary, secondary and tertiary treatment prior to being released to the municipal drainage network (Al-Najar, 2018). Furthermore, the advanced age of the wastewater drainage networks

and the lack of appropriate maintenance causes frequent clogging and flooding incidents, particularly where wastewater and storm water networks are not separated. Well-designed water drainage system exists in 38% of the assessed health care facilities, while in 48%, the system could carry contamination outside the health care settings (UNICEF & WW-GVC, 2019).

3. Objectives of the Pilot Study

To recall from Section 1, the working hypothesis of this WASH-HCF-AMR study is that AMR pathogens can be transmitted at health care facilities through WASH services, and can leave the hospital through its wastewater effluent. Figure 4 illustrates the potential transmission of AMR to/from HCF through water and sanitation.

The **general objective** of this pilot study is to establish the extent to which the original hypothesis may be further investigated.

The **specific objectives** were:

- To investigate the occurrence of antibiotic resistance bacteria in water samples collected from drinking and domestic water at healthcare facilities;
- To investigate the occurrence of antibiotic resistance bacteria in healthcare wastewater effluent; and
- To investigate the occurrence of antibiotic resistance bacteria in surface swabs collected from hygiene facilities in healthcare facilities.

The study objectives were met investigation of:

i) the presence of AMR bacteria in samples collected from water, wastewater and hygiene facilities at health care settings in Gaza; and

ii) the presence of ESBL producing *E. coli* and carbapenem resistant Gram-negative bacteria (GNB), methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin resistant Enterococci (VRE) in water and wastewater samples, and surface swabs collected from two hospitals.

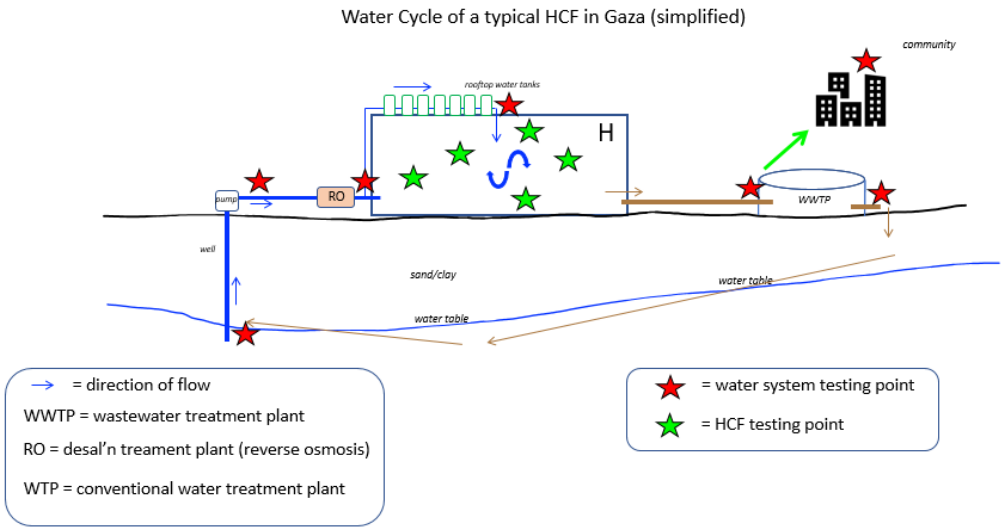

Figure 4 : Potential transmission of antimicrobial resistant bacteria

4. Methodology

3.1 Study design

A hospital-based cross-sectional study was conducted to detect and identify antimicrobial resistance bacteria in water and wastewater samples in addition to surface swabs (hygiene facility). The design, sampling, testing, and analysis were led by Reem Abu Shomar, with the direct supervision of Prof. Abdelraouf A. Elmanama. Consultation on the selection of PCR primers, provision of positive controls for some AMR genes and support to interpretation of results were provided by Dr Antoine Abu Fayyad. Defining the concept of the research, ongoing guidance (in line with the WAMREW 1st workshop theme), commenting and provision of constructive feedback on study reports were provided by Professor Mark Zeitoun and Dr. Ghassan Abu-Sittah. Appreciated verbal feedback during the 1st WAMREW workshop was received from Prof. Paul R Hunter, Dr. Mirko Winker, Eng. Federico Sittaro, Dr. Jo Geere, and Dr. Nassim Achi. The research process was managed by Dr. Zahy Abdul Sater, and Dr. Theresa Farhat. Also appreciated the mentoring provided by Dr. Aula Abbara and coordination made by Mr. Tarek Kishawi under the CREEW fellowship.

3.2 Setting

The study was carried out in two large governmental hospitals in Gaza Strip; Al-Shifa and the EGH. As Al Shifa Hospital does not have a wastewater treatment facility its effluent is transmitted untreated to the municipal drainage network, and to Al-Sheik Ejleen wastewater treatment plan. The European Hospital Complex has its own wastewater treatment facility which generally provides secondary-level treatment before discharge to the municipal drainage network and Al-Sheik Ejleen wastewater treatment plant.

Ethical considerations & procedures

Permission to undertake the study in governmental hospitals was provided by the MOH. In addition, the confidentiality of all private information was assured through coding while sampling and entering data.

3.3 Sampling

Random samples from water, wastewater, soap, and surface swabs (n=345) were collected from the two hospitals; 50.4 % (n=174) of samples were collected from the European Gaza Hospital (EGH) and 47.5% (n=164_ were collected from Al Shifa hospital. The remaining portion (2% (n=7)) was collected from Gaza WWTP. The samples were collected in May and June, 2020.

At both hospitals, 45.8% of samples were collected from male surgical wards, 45.2% were collected from the female surgical wards. The samples collected from water and wastewater facilities located outside the surgical wards represented 9% of the total samples.

About two thirds (62.6%) of the samples were swabs collected from hygiene facilities in the surgical wards at both hospitals. 19.4% were water samples collected from different sites at the hospitals; of these 11.6% were samples collected from the liquid soap available at the patients' and nurses' handwashing facilities in the surgical wards, and 6.4% were wastewater samples. The type of collected samples and their percentage is shown in figure 5.

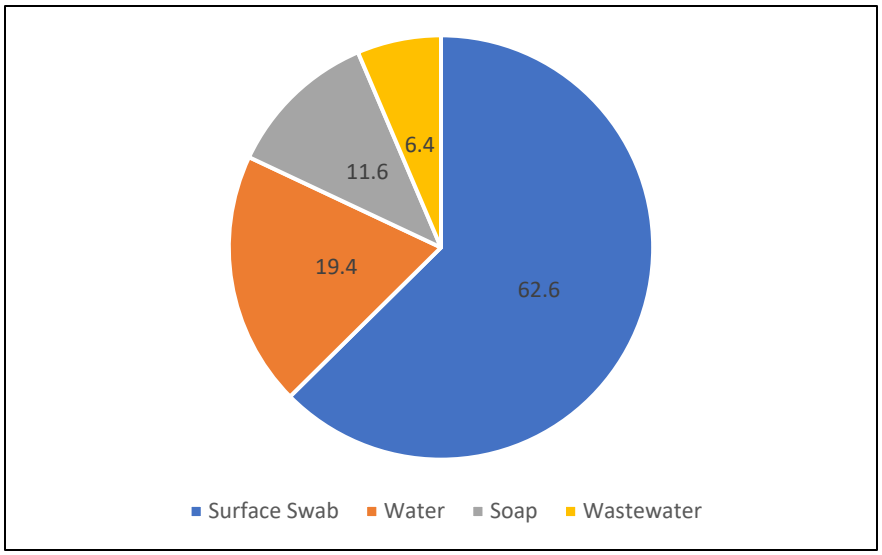

Figure 5: Sample Distribution of samples taken in the two hospitals (n=345)

About two-thirds (62%) of the sample set (including water, swabs and soap) were from bathrooms and 28.1% were from patients' and nurses' rooms in both hospitals. More than half of the end users of tested WASH facilities (58.1%) were in-patient or their accompanies. 24.2% were nursing or medical staff and 17.7% were septic patient or their accompanies.

Three-quarters (74.6%) of the collected water samples were from tap water, 6% were from wells, 16.5% from RO desalination supply chain, and 3% from municipal water supply point. From both hospitals, 11.1% surface swab samples were collected from each of the following sites; bathroom's inner door handle, toilet seat area, toilet bidet, and toilet foot rest area. 18.5% of the swabs were collected from each of the following sites: the tap mouth, tap handle, and liquid soap bottle hand.

Both water and wastewater samples were collected in accordance with the World Health Organization guidelines. Samples were collected by a qualified technician in sterile polyethylene bottles, labelled properly, and transported to the microbiology laboratory of the Islamic University of Gaza in an Ice box within two hours of collection for testing according to standard procedures (*see Annex A*). The methodology for direct laboratory testing and investigation of resistance bacteria followed the schematic of Figure 6.

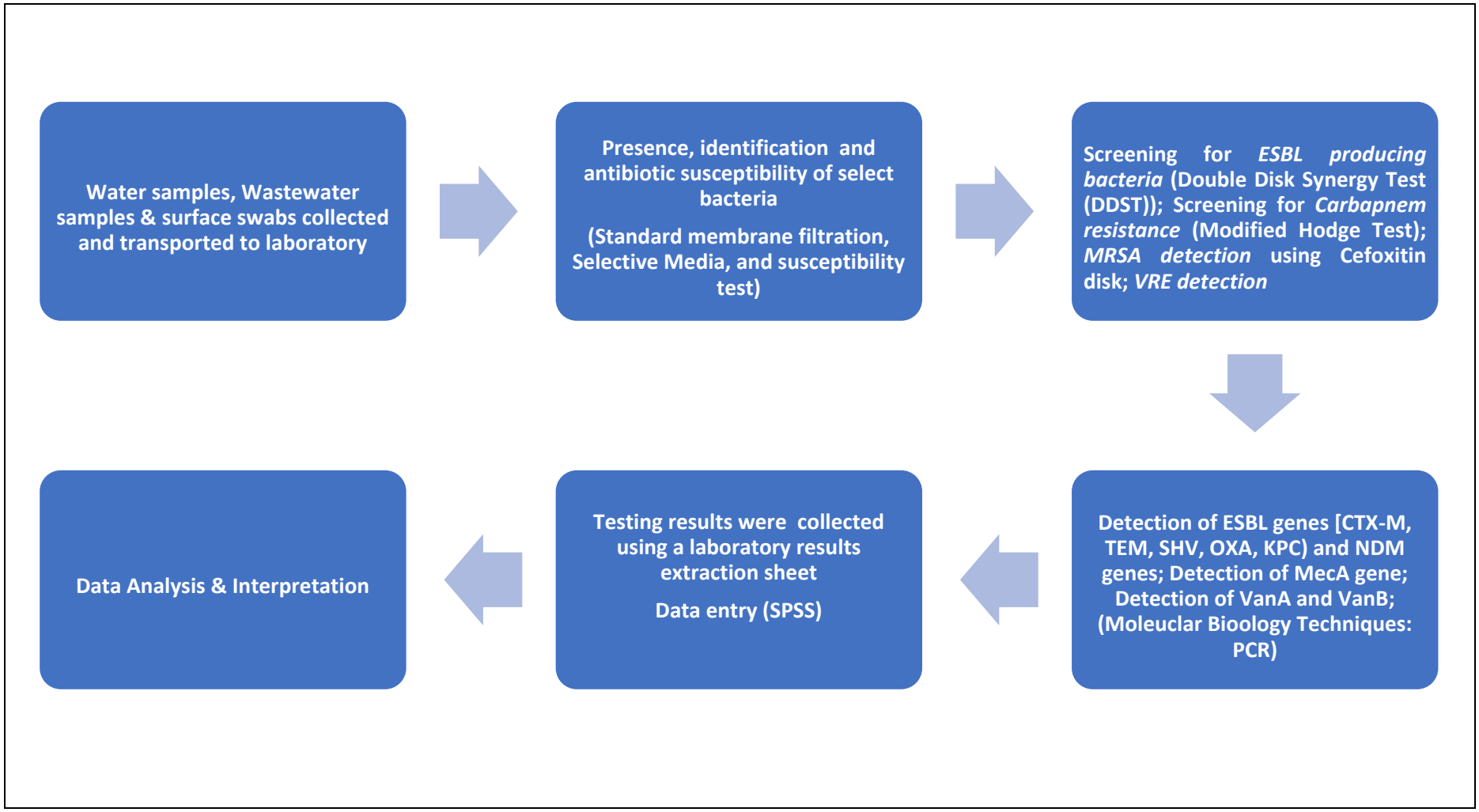

Figure 6. Summary flow chart of the methodology for laboratory direct testing and investigation of resistance bacteria

3.4 Data analysis

Data collected in an extraction sheet was summarized, tabulated, and analyzed using Statistical Package for Social Sciences (SPSS) software and the statistical significance was tested at P value of 0.05 where possible.

3.5 Study limitations

- The cross-sectional study design provides a snapshot of the outcome at specific point of time
- The grab wastewater sample reflects the results at one point of time.
- Resistance gene-negative isolates (genotyping) doesn't rule out the antibiotic resistance
- Antibiotic Sensitivity testing (phenotyping) reflects the ability of antibiotic to inhabit bacterial growth in vitro

5. Results

4.1 Microbial growth and enumeration

The overall percentage of positive cultures for samples collected from both hospitals without including the wastewater samples (n=323) was 34.1%. The slight difference (35.6% in Al-Shifa, 32.5% in EGH) was statistically insignificant. From the total number of collected samples including the wastewater samples (n=345), there were 46 out of 216 (21.3%) positive swab samples; 64 out of 67 (95.5%) positive water samples; and 22 out of 22 (100%) positive wastewater samples. Soap samples (n=40) were totally negative for bacterial growth.

The percentage of growth on selective media per sample type is shown below in Table (1).

Table 1: Bacterial Growth on Selective Media/Sample Type

	Swab				Water				Wastewater			
	Positive		Negative		Positive		Negative		Positive		Negative	
	N	%	N	%	N	%	N	%	N	%	N	%
m-Endo Agar	23	10.6%	193	89.4%	37	55.2%	30	44.8%	21	95.5%	1	0.50%
Cetrimide Agar	26	12.0%	190	88.0%	41	61.2%	26	38.8%	15	68.2%	7	31.8%
m-Enterococcus Agar	6	2.8%	210	97.2%	22	32.8%	45	67.2%	14	63.6%	8	36.4%
Manitol Salt Agar	26	12.0%	190	88.0%	49	73.1%	18	26.9%	12	54.5%	10	45.5%

The bacterial growth on each selective media for water samples were enumerated and the results are presented in the Table (2). It is worth mentioning that no bacterial growth is permitted, according to WHO guidelines and Palestinian standards. More than 100 CFU/ml bacterial growth was detected in about 14% of enumerated bacteria on m-Endo *Agar*; 29% of enumerated on Cetrimide Agar; 12.5% of enumerated on m-*Enterococcus Agar*; and 24.5% of enumerated on Manitol Salt Agar.

Table 2: Bacteria Enumeration for Water Samples on Selective Media

Bacteria Count (CFU/ml)		m-Endo Agar	Cetrimide Agar	m-Enterococcus agar	Manitol Salt Agar
Less or equal 5	**N**	31	14	12	11
	%	54.4	34.1	40.0	22.4
6-100	**N**	18	15	9	26
	%	31.6	36.6	37.5	53.1
More than 100	**N**	8	12	3	12
	%	14.0	29.3	12.5	24.5

The variation in bacterial growth between the two hospitals is shown in table 3, for swab and water samples. The growth on m-Endo Agar was positive in 30.5% of tested samples in AL Shifa Hospital compared to 12.1% in EGH (considered statistically significant ($p < 0.001$). The variation in bacterial growth percentage between AL Shifa and EGH hospital on Cetrimide Agar, m-Enterococcus Agar, and Manitol Salt Agar were not statistically significant.

Table 3: Bacterial Growth per Hospital

Microorganism	Hospital Name				Total	
	Al-Shifa		EGH			
	N	%	N	%	N	%
m-Endo Agar						
Positive	50	30.5	21	12.1	71	21
Negative	114	69.5	153	87.9	267	79
Cetrimide Agar						
Positive	41	25	35	20.1	76	22.5
Negative	123	75	139	79.9	262	77.5
m-Enterococcus Agar						
Positive	23	14.0	19	10.9	42	12.4
Negative	141	86.0	155	89.0	296	87.6
Manitol Salt Agar						
Positive	41	25	41	23.6	82	24.3
Negative	123	75	133	76.4	256	75.7

4.2 ESBL and Modified Hodge Testing Results for Enterobacteriacea

The percentage of positive Modified Hodge Testing (MHT) and ESBL producing *Enterobacteriacea* are shown in Figure 7. Twenty two percent of identified Enterobacteriacea were positive for ESBL and fourteen percent were positive for MHT.

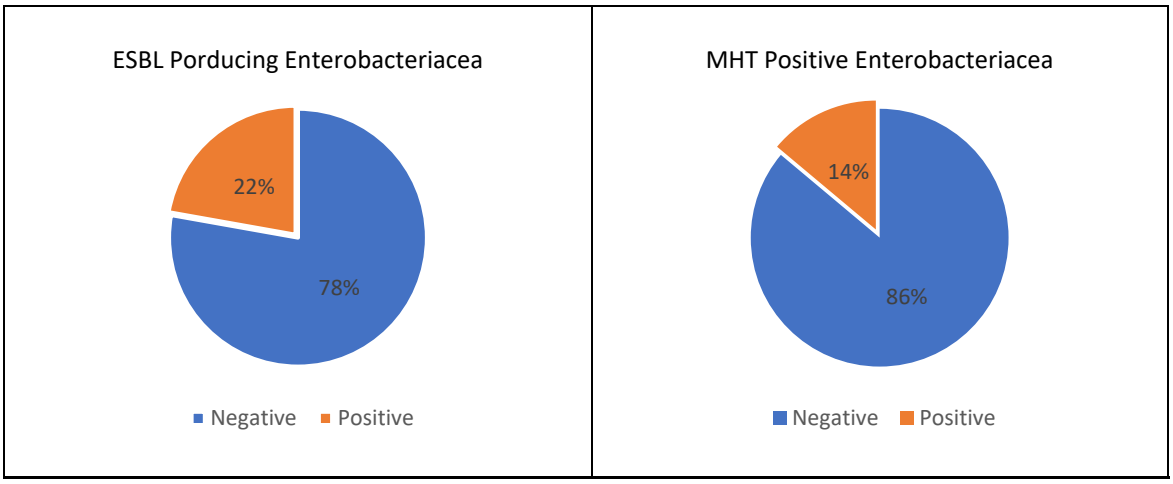

Figure 7: ESBL and MHT Results

4.3 Antibiotic resistance profile of the identified bacteria per sample type

The antibiotic resistance profile for the identified bacteria are shown in Tables 4 – 7, listed tables under four main groups; (1) *Enterobacteriaceae* , (2) *Pseudomonas*, (3) *Staphylococcus*, and (4) *Enterococcus*.

4.3.1 Antibiotic resistance profile for Enterobacteriaceae

Table (4) shows that Enterobacteriaceae isolates from swab samples were completely resistant (100%) to Pipracillin-Tazopactam, Amikacin, Ceftazidime, and Ceftriaxone, moreover, 70% of the isolated bacteria were resistant to Imipenem While more than two thirds of isolated Enterobacteriaceae from water and wastewater samples showed resistance patterns for; Amikacin (75.0% & 94.4%), Ceftazidime (75.0% & 94.4%, Ceftriaxone (75.0% & 83.3%), and Imipenem (62.5% & 66.7%).

The variation of antibiotic resistance patterns among sample types for Enterobacteriaceae were statistically insignificant with the exception of resistance patterns related to Cefepime and Chloramphenicol antibiotics.

4.3.2 Antibiotic Resistance Profile for Pseudomonas

As shown in Table (5), more than two thirds of *Pseudomonas* (77.8%) isolated from swab samples were resistant to Aztreonam, Meropenem, Ceftazidime, & Ceftazidime-avibactam and (66.7%) of the isolates was resistant to Piperacillin.

For water samples, more than two thirds of the isolates was resistant to Aztreonam (69.2%), Ceftazidime-avibactam (69.2%), Piperacillin (61.5%), Meropenem (61.5%), & Cefepime (61.5%).

For wastewater samples, the isolates showed complete resistance (100%) to Aztreonam, Cefepime and Gentamicin. Half (50%) resistance were shown for Piperacillin, Meropenem, Ceftazidime, & Ceftazidime-avibactam.

The variation of antibiotic resistance patterns among sample types for *Pseudomonas* were statistically insignificant in all selected antibiotics.

Table 4: Antibiotic Resistance Profile for Enterobacteriaceae per Sample Type

	Sample Type																			
	Swab						Water						Wastewater						X2	p
	S		I		R		S		I		R		S		I		R			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
Amikacin	-	-	-	-	10	100.0	2	25.0	-	-	6	75.0	1	5.6	-	-	17	94.4	4.0	1.35
Cefepime	4	40.0	-	-	6	60.0	8	100.0	-	-	-	-	14	77.8	2	11.1	2	11.1	13.57	0.009
Cefoxitin	4	40.0	2	20.0	4	40.0	3	37.5	1	12.5	4	50.0	8	44.4	3	16.7	7	38.9	0.397	0.98
Ceftazidime	-	-	-	-	10	100.0	2	25.0	-	-	6	75.0	1	5.6	-	-	17	94.4	4.00	0.14
Ceftriaxone	-	-	-	-	10	100.0	2	25.0	-	-	6	75.0	3	16.7	-	-	15	83.3	2.56	0.28
Chloramphenicol	6	60.0	-	-	4	40.0	8	100.0	-	-	-	-	17	94.4	-	-	1	5.6	8.04	0.018
Ciprofloxacin	2	20.0	4	40.0	4	40.0	6	75.0	1	12.5	1	12.5	11	61.1	2	11.1	5	27.8	7.25	0.12
Fosfomycin	6	60.0	1	10.0	3	30.0	5	62.5	-	-	3	37.5	7	38.9	5	27.8	6	33.3	3.90	0.42
Gentamicin	5	50.0	1	10.0	4	40.0	6	75.0	1	12.5	1	12.5	11	61.1	2	11.1	5	27.8	1.69	0.79
Imipenem	-	-	3	30.0	7	70.0	3	37.5	-	-	5	62.5	3	16.7	3	16.7	12	66.7	6.12	0.19
Levofloxacin	8	80.0	-	-	2	20.0	8	100.0	-	-	-	-	16	88.9	-	-	2	11.1	1.8	0.41
Nitrofurantoin	6	60.0	4	40.0	-	-	5	62.5	-	-	3	37.5	12	66.7	2	11.1	4	22.2	8.34	0.08
Pipracillin-Tazopactam	-	-	-	-	10	100.0	3	37.5	1	12.5	4	50.0	4	22.2	4	22.2	10	55.6	7.99	0.092

S, susceptible; I, intermediate; R, resistance.

Table 5: Antibiotic Resistance Profile for *Pseudomonas* per Sample Type

	Sample Type																			
	Swab						Water						Wastewater						X^2	*p*
	S		I		R		S		I		R		S		I		R			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
Aztreonam	2	22.2	-	-	7	77.8	4	30.8	-	-	9	69.2	-	-	-	-	2	100.0	0.93	0.63
Cefepime	2	22.2	2	22.2	5	55.6	4	30.8	1	7.7	8	61.5	2	100.0	-	-	-	-	5.41	0.25
Ceftazidime	2	22.2	-	-	7	77.8	5	38.5	2	15.4	6	46.2	1	50.0	-	-	1	50.0	3.23	0.52
Ceftazidime-avibactam	2	22.2	-	-	7	77.8	4	30.8	-	-	9	69.2	1	50.0	-	-	1	50.0	0.65	0.72
Gentamicin	3	33.3	3	33.3	3	33.3	6	46.2	6	46.2	1	7.7	2	100.0	-	-	-	-	5.1	0.28
Imipenem	3	33.3	2	22.2	4	44.4	9	69.2	4	30.8	-	-	1	50.0	1	50.0	-	-	8.397	0.08
Meropenem	2	22.2	-	-	7	77.8	4	30.8	1	7.7	8	61.5	1	50.0	-	-	1	50.0	1.61	0.81
Piperacillin	3	33.3	-	-	6	66.7	5	38.5	-	-	8	61.5	1	50.0	-	-	1	50.0	0.21	0.90

S, susceptible; I, intermediate; R, resistance.

4.3.3 Antibiotic resistance profile for Enterococcus

As shown in Table (6), irrespective to sample type, all Enterococcus isolates were completely resistant to Penicillin (100%). 80% of Enterococcus isolates from surface swab's samples were resistance to Tetracycline, Vancomycin, Gentamicin, & Linezolid.

For water samples, 100% of Enterococcus isolates were resistant to Vancomycin, Gentamicin, Linezolid, & Penicillin. In addition, more than two thirds (66.7%) of isolates were resistant to Tetracycline, Ciprofloxacin, & Fosfomycin.

Like the swab and water samples, wastewater samples were found to be 100% resistant to Penicillin. For wastewater samples, and when compared to other types of samples, the isolates showed relatively lower percentage of resistant (53.8%) for Tetracycline, Vancomycin, & Linezolid. 46.2% for Gentamicin, 15.4% for Nitrofurantoin, 46.2% for Ciprofloxacin, 15.4% for Fosfomycin.

The variation of antibiotic resistance patterns among Enterococcus isolates between sample types were found to be statistically insignificant

4.3.4 Antibiotic resistance profile for S. aureus

As shown in Table (7), *S. aureus* isolates were sensitive for most of the antibiotics, with the exception of Penicillin (more than 96% of isolates were resistant to Penicillin).

Surface swabs samples were found to be resistance for Cefoxitin (14.3%), Chloramphenicol (14.3%), Erythromycin (42.3%) & Penicillin (100.%).

For water samples, 96.6% of isolates were resistant to Penicillin, 42.9% 13.8% were resistance to Cefoxitin and Tetracycline.

Resistance were reported for wastewater samples with regard to penicillin (100%), Levofloxacin (33.3%), Linezolid (33.3%), Vancomycin (33.3%) and Cefoxitin (33.3%).

The variation of antibiotic resistance patterns among *S. aureus* between sample types were statistically insignificant.

Table 6: Antibiotic Resistance Profile for *Enterococci* per Sample Type

	Sample Type																			
	Swab						Water						Wastewater						X^2	*p*
	S		I		R		S		I		R		S		I		R			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
Ciprofloxacin	3	60.0	1	20.0	1	20.0	1	33.3	-	-	2	66.7	5	38.5	2	15.4	6	46.2	2.058	0.725
Fosfomycin	2	40.0	1	20.0	2	40.0	-	-	1	33.3	2	66.7	8	61.5	3	23.1	2	15.4	4.71	0.318
Gentamicin	1	20.0	-	-	4	80.0	-	-	-	-	3	100.0	3	23.1	4	30.8	6	46.2	4.64	0.327
Linezolid	1	20.0	-	-	4	80.0	-	-	-	-	3	100.0	6	46.2	-	-	7	53.8	2.862	.239
Nitrofurantoin	2	40.0	1	20.0	2	40.0	1	33.3	1	33.3	1	33.3	9	69.2	2	15.4	2	15.4	2.355	0.671
Penicillin	-	-	-	-	5	100.0	-	-	-	-	3	100.0	-	-	-	-	13	100.0	-	-
Tetracycline	1	20.0	-	-	4	80.0	1	33.3	-	-	2	66.7	5	38.5	1	7.7	7	53.8	1.396	0.845
Vancomycin	2	40.0	-	-	3	60.0	-	-	-	-	3	100.0	6	46.2	-	-	7	53.8	2.122	0.33

S, susceptible; I, intermediate; R, resistance.

Table 7: Antibiotic Resistance Profile for *S. aureus* per Sample Type

	Swab						Water						Wastewater						X^2	*p*
	S		I		R		S		I		R		S		I		R			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
Cefoxitin	6	85.7	-	-	1	14.3	25	86.2	-	-	4	13.8	2	66.7	-	-	1	33.3	0.805	0.669
Chloramphenicol	5	71.4	1	14.3	1	14.3	20	69.0	6	20.7	3	10.3	3	100.0	-	-	-	-	1.499	0.827
Clindamycin	4	57.1	3	42.9	-	-	11	37.9	6	20.7	12	41.4	3	100.0	-	-	-	-	8.58	0.72
Doxycycline	7	100.0	-	-	-	-	27	93.1	-	-	2	6.9	3	100.0	-	-	-	-	0.73	0.695
Erythromycin	3	42.9	1	14.3	3	42.9	7	24.1	13	44.8	9	31.0	1	33.3	2	66.7	-	-	3.79	0.435
Levofloxacin	7	100.0	-	-	-	-	27	93.1	-	-	2	6.9	2	66.7	-	-	1	33.3	3.39	0.18
Linezolid	7	100.0	-	-	-	-	26	89.7	-	-	3	10.3	2	66.7	-	-	1	33.3	2.54	0.281
Nitrofurantoin	7	100.0	-	-	-	-	27	93.1	-	-	2	6.9	3	100.0	-	-	-	-	0.73	0.695
Penicillin	-	-	-	-	7	100.0	1	3.4	-	-	28	96.6	-	-	-	-	3	100.0	0.354	0.838
Rifampicin	6	85.7	1	14.3	-	-	24	82.8	5	17.2	-	-	3	100.0	-	-	-	-	0.63	0.73
Tetracycline	4	57.1	3	42.9	-	-	22	75.9	3	10.3	4	13.8	2	66.7	1	33.3	-	-	5.41	0.247
Vancomycin	4	85.7	2	28.3	1	14.3	25	86.2	3	10.3	1	3.4	2	66.7	-	-	1	33.3	6.18	0.186

S, susceptible; I, intermediate; R, resistance.

4.4 Molecular testing of AMR

Table 8 presents the results of AMR genes among each type of isolated bacteria. The prevalence of ESBL genes among *Enterobacteriaceae* isolates were: 11.1% for NDM, 25% OXA, 19,4% SHV, 2.8% KPC, 66.7% TEM, 41.7% blaCTXM, and 5.6% blaCTXM-3. For Carbapenem resistant gene (MDM), the prevalence among Enterobacteriaceae was 11.1% and among *Pseudomonas* was 12.5%. Vancomycin resistance gene (VAN_A) was positive in 4.8% of Enterococcus isolates and (VAN_B) was negative in Enterococcus and *S. aureus* isolates. For the Methicillin resistant gene (MEC), it was positive in 51.3% of *S. aureus* isolates.

Table 8: Prevalence of AMR Resistant Genes among each Microorganism

		NDM	OXA	SHV	KPC	TEM	blaCTXM	blaCTXM-3	MEC	VAN_A
		N	N	N	N	N	N	N	N	N
		%	%	%	%	%	%	%	%	%
Microorganisms	*Enterobacteriaceae*	4	9	7	1	24	15	2	NA	NA
		11.1	25.0	19.4	2.8	66.7	41.7	5.6		
	Pseudomonas	3	NA	NA	NA	NA	NA	NA	NA	NA
		12.5								
	Enterococcus	NA	NA	NA	NA	NA	NA	NA	NA	1
										4.8
	S aureus	NA	NA	NA	NA	NA	NA	NA	20	0
									51.3	0

NA= Not applicable

6. Conclusion

To recall, the objective of this WASH-HCF-AMR pilot study is to establish the extent to which the original hypothesis may be further investigated. The working hypothesis of this WASH-HCF-AMR study is that AMR pathogens can be transmitted at health care facilities through WASH services, and can reach the community through wastewater.

The results revealed a number of headline findings that may be worth further investigation:

1. Alarmingly high levels of bacterial contamination: 34.1% of the total sample set excluding the wastewater samples (n=323). Over 1/5th of the swab samples (46) and 95% of the water samples (65) were positive. As would be expected, 100% of the wastewater samples (22) were also positive.
2. Of the total positive microbial growth, 35.7% *on* m-Endo Agar, 25.2% on Manitol Salt Agar, 23.8% on Cetrimide Agar; and 12.2% on m-Enterococcus Agar.
3. Of the isolated *Enterobacteriaceae*, twenty-two percent of identified *Enterobacteriaceae* was positive for ESBL and fourteen percent was positive for MHT.
4. The antibiotic resistance profiles demonstrated elevated levels of resistance across the bacteria, and often total resistance.
    a. 100% of *Enterobacteriaceae* isolated from swab samples were found to be resistant to Pipracillin-Tazopactam, Amikacin, Ceftazidime, and Ceftriaxone. Over 2/3 of water and wastewater samples found resistant to Amikacin, Ceftazidime Ceftriaxone, and Imipenem.
    b. Over two-thirds of *Pseudomonas* (77.8%) isolated from swab samples were resistant to Aztreonam, Meropenem, Ceftazidime, & Ceftazidime-avibactam, and two-third (66.7%) of the isolates was resistant to Piperacillin. Approximately two-thirds of water samples found to be resistant to Aztreonam, Ceftazidime-avibactam, Piperacillin, Meropenem, & Cefepime. 100% of wastewater samples found to be resistant to Aztreonam, and sensitive to Cefepime and Gentamicin.
    c. *All Enterococcus* isolates were found to be completely resistant to penicillin, whether surface swabs, water or wastewater samples. 80% of isolates from surface swabs were found to be resistant to Tetracycline, Gentamicin, & Linezolid. 100% of Enterococcus isolates in water samples were found to be resistant to Vancomycin, Gentamicin, Linezolid, & Penicillin (and over two-thirds (66.7%) resistant to Tetracycline, Ciprofloxacin, & Fosfomycin).

Approximately half of the wastewater samples found to be resistant to Tetracycline, Vancomycin, & Linezolid.

d. Though *S. aureus* isolates were found to be the most sensitive for most of the antibiotics, still more than 96% were resistant to Penicillin. In surface swab samples, intermediate resistant was reported for Tetracycline (42.9%) & Clindamycin (42.9%). Intermediate resistance were reported for water and wastewater samples with regard to Erythromycin; 44.8% & 66.7 respectively. Approximately 13.8% of *S. aureus* isolated from water samples were resistant to Cefoxitin.

5. Genotyping revealed that, ESBL genes among Enterobacteriaceae isolates for OXA, SHV, KPC, TEM, blaCTXM, and blaCTXM-3 were detected in the collected samples from different sources. Carbapenem resistant gene (NDM) was found in 11.1% of Enterobacteriaceae isolates and 12.5% in Pseudomonas. 4.8% of Enterococcus isolates were positive for Vancomycin resistance gene (VAN_A) and 51.3% of S. aureus isolates were positive for Methicillin resistant gene (MEC).
6. In relation to other studies on AMR: i) The absence of microbiological contamination in samples collected from liquid soap in this study contradict findings from other studies in Gaza (Altaher AM, Abdul Ghafoor ES, Amudi WI, & al, 2016; Salama, 2017). The enhanced monitoring and quality assurance of the liquid soap provided by the outsourced service provider might explain those findings. Ii) The existence of AMR bacteria in medical hospital wastewater has been mentioned in previous studies however, this pilot study was unique in terms of investigating the presence of AMR genes in water, wastewater and surface swabs collected from HCFs in Gaza.
7. Policy relevance based on the preliminary results: The spread of AMR bacteria is known to be influenced by infection control practices, access to clean water and sanitation, proper diagnosis and treatment with quality assured antimicrobials (Holmes et al., 2016). The results of this pilot study highlighted the AMR burden attributed to unsafe WASH services at healthcare settings. The results indicate the need to consider the safety of WASH service provided at HCFs as an immediate concern in the fight against the spread of AMR. Put another way, investment in improving the IPC practices and the management of antibiotic use on its own will not likely be effective, if WASH services are not also considered. A comprehensive multidimensional efforts that covers the environmental aspects including access to safe WASH services is curtail to prevent the spread of AMR bacteria and genes. The results also indicated the importance of setting priorities about investing in AMR prevention through improved WASH in the national AMR polices and plans.

8. Further research: The findings of this pilot study brought attention to the role of unsafe WASH services in AMR transmission, for which further research is required:

i) The elevated levels of resistance in the bulk of samples suggests a pressing need for more investigation into the root causes of such burden attribute to unsafe WASH service and how this burden would be mitigated.

ii) More investment is required to ensure proper WASH services and interventions at healthcare settings including proper water disinfection and medical wastewater treatment.

iii) Additional molecular investigation such as Whole Genome Sequencing (WGS) can be undertaken to identify whether the isolated AMR species belonging to the same species of AMR bacteria in the MENA region.

7. References

Abu Sitta, G., Dewachi, O., Nguyen, V., & Whittall, J. (2016). Conflict medicince—a manifesto. *MSF Analysis: Reflections on Humanitarian Action*.

Al Laham, N. A. (2012). Distribution and antimicrobial resistance pattern of bacteria isolated from operation theaters at Gaza strip. *Journal of Al Azhar University-Gaza (Natural Sciences), 14*(1), 19-34.

Al-Najar, H., Ghourab, A., Eid, R., & Farhouda, H. (2018). Medical Wastewater Characterization in the Gaza Strip: Al-Shifa Medical Complex as a Case Study. *Health Scope*, *7*(3).

Altaher AM, Abdul Ghafoor ES, Amudi WI, & al, e. (2016). Comparative Identification of Bacterial Quality in liquid Soap between Nasser and European Gaza Hospitals, Khanyounis Governorate. *Asian Journal of Pharmacy, Nursing and Medical Sciences, 4(5)*, 77–83.

Bouzid, M., Cumming, O., & Hunter, P. R. (2018). What is the impact of water sanitation and hygiene in healthcare facilities on care seeking behaviour and patient satisfaction? A systematic review of the evidence from low-income and middle-income countries. *BMJ global health, 3*(3), e000648.

CMWU. (2018). Costal Municipalities Water Utilities: Annual Report 2017. Palestine, Gaza.: Costal Municpalities Water Utilities.

Dewachi, O., Skelton, M., Nguyen, V.-K., Fouad, F. M., Sitta, G. A., Maasri, Z., & Giacaman, R. (2014). Changing therapeutic geographies of the Iraqi and Syrian wars. *The Lancet, 383*(9915), 449-457.

Elmanama, A. A., Alyazji, A. A., & Abu-Gheneima, N. (2011). Antibacterial, antifungal and synergistic effect of Lawsonia inermis, Punica granatum and Hibiscus sabdariffa. *Antibacterial, antifungal and synergistic effect of Lawsonia inermis, Punica granatum and Hibiscus sabdariffa, 7*.

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., . . . Piddock, L. J. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet, 387*(10014), 176-187.

Hujier, A., & Saleem, N. (2006). Detection of methicillin-resistant Staphylococcus aureus in nosocomial infections in Gaza Strip. *Detection of Methicillin-Resistant Staphylococcus aureus in Nosocomial Infections in Gaza Strip*.

Kanapathipillai, R., Malou, N., Hopman, J., Bowman, C., Yousef, N., Michel, J., . . . Mills, C. (2019). Antibiotic resistance in conflict settings: lessons learned in the Middle East. *JAC-Antimicrobial Resistance, 1*(1), dlz002.

Mendelson, M., & Matsoso, M. P. (2015). The World Health Organization global action plan for antimicrobial resistance. *SAMJ: South African Medical Journal, 105*(5), 325-325.

MoH. (2017). Health Annual Report, Palestine 2016. *Ministry of Health, Palestine*.

O'Neill, J. (2016). Tackling drug resistance infections globally: final report and recommendations: the review on antimicrobial resistance. Government of the United Kingdom, London, United Kingdom.

OCHA. (2019). Increased electricity supply improves access to water and sanitation in Gaza *Monthly Humanitarian Bulletin/August 2019*. oPt.

PCBS. (2018a). Palestinians at the End of 2018. Ramallah-Palestine.: Palestinian Central Bureau of Statistics.

PCBS. (2018b). *Population, Housing and Establishments Census 2017: Census Final Results-Summary-2017*. Ramallah-Palestine: Palestinian Central Bureau of Statistics.

PWA. (2015). Survey of Private and Public Brackish Desalination Plants in Gaza Strip Gaza Strip, Palestine.

PWA. (2018). *Water Resources Status Report*. Palestine: Palestinian Water Authority.

PWA. (2019). Water Safety for Public Health Status Joint Report. Gaza: Palestinian Water Authority.

Salama, A. S. A. (2017). Microbiological Quality of Soaps and Efficacy of Antiseptics and Disinfectants Used in Hospitals in Gaza-Palestine. *Microbiological Quality of Soaps and Efficacy of Antiseptics and Disinfectants Used in Hospitals in Gaza-Palestine*.

Shomar, R. A., Abdelatif, M., & Kishawi, Y. (2017). Visualising the spatial distribution of diarrheal disease using the geographical information system: a WASH perspective. *Int. J. Global Environmental Issues, 16*(1/2), 3.

Tayh, G., Ben Sallem, R., Ben Yahia, H., Gharsa, H., Klibi, N., Boudabous, A., & Ben Slama, K. (2017). First report of extended-spectrum β-lactamases among clinical isolates of Klebsiella pneumoniae in Gaza strip, Palestine. *Microbial Drug Resistance, 23*(2), 169-176.

UNICEF, & WW-GVC. (2019). WASH Assessmetn and Surveillance System in Health Faciliteis in the Gaza Strip. Palestine, Gaza Strip.

Watson, J., D'Mello-Guyett, L., Flynn, E., Falconer, J., Esteves-Mills, J., Prual, A., . . . Cumming, O. (2019). Interventions to improve water supply and quality, sanitation and handwashing facilities in healthcare facilities, and their effect on healthcare-associated infections in low-income and middle-income countries: a systematic review and supplementary scoping review. *BMJ global health, 4*(4), e001632.

Watts, S., Siddiqi, S., Shukrullah, A., Karim, K., & Serag, H. (2007). Social Determinants of Health in Countries in Conflict: The Eastern Mediterranean Perspective: Cairo, Egypt: Commission on Social Determinants of Health, Health Policy and ….

WHO. (2008). *Closing the gap in a generation: health equity through action on the social determinants of health*: World Health Organization.

WHO. (2019). WASH in health care facilities: global baseline report 2019.

WHO, & UNICEF. (2016). Water, Sanitation and Hygiene (WASH) in Health Care Facilities-Global Action Plan: WHO/UNICEF.

WHO, & UNICEF. (2019). WASH in health care facilities: Global Baseline Report 2019. Geneva.

World Bank. (2017). Towards Water Security for Palestinians: A diagnostic of improvements needed in water and sanitation services in the West Bank & Gaza: World bank.

Zeitoun, M., & Abu Sitta, G. (2018). Gaza now has a toxic 'biosphere of war'that no one can escape.

8. Annex A: Laboratory methods

7.1 Microbiological analysis

7.1.1 Microbiological analysis of water sample:

The membrane filtration technique was used, in which 100 ml of the sample poured aseptically into a sterile filtration assembly containing a sterile membrane filter (a 0.45 um Millipore filter). A vacuum was applied and the sample was drawn through the membrane filter. All organisms were retained on or within the membrane filter, which is then transferred to appropriate counting/isolation culture Media in a petri dish.

Each plate was incubated at the appropriate temperature, and examined for growth. For water samples, colonies were counted and expressed as CFU/ml. A representative colony from each plate was sub-cultured, identified and stored in 40 glycerol/60 Brain Heart Infusion Broth (BHIB) for further Antimicrobial Susceptibility Testing (AST) & molecular testing.

7.1.1 Microbiological analysis of wastewater samples

After mixing the collected sample, a sterile loop was used to streak four different plates: 1) m-Endo agar media for *Enterobacteriacae*, 2) Cetrimide agar (CA) for *Pseudomonas aeruginosa*, 3) Manitol salt agar (MSA) for *S. aureus* and 4) m-Enterococcus agar for *Enterococcus* isolation. Plates were incubated for 24-48 hours. Each plate showed growth was used as a source of one colony that was confirmed and stored in 40 glycerol/60 BHIB for further AST & Molecular testing.

7.1.2 Identification of isolates

Biochemical testes was done by colony morphology and characteristic growth, Gram stain, motility test, triple sugar iron agar and pattern of biochemical profile (oxidase, catalase, urease and citrate) were used to confirm the identity of Gram-negative isolates. While catalase, coagulase, bile esculin, salt tolerance and other conventional biochemical tests were used to identify Gram-positive isolates.

7.1.3 Antimicrobial susceptibility Tests

The growth of each of the tested isolates was standardized using colony suspension method. Growth suspension was matched with 0.5 McFarland standards to give a resultant concentration of about 1.5 × 108 cfu/mL. The antimicrobial susceptibility testing was determined using the modified Kirby–Bauer diffusion technique, by swabbing the Mueller-Hinton agar (MHA) plates with the resultant BHIB suspension of each isolate, different antimicrobial disks were carefully placed onto the surface of the inoculated plates. The antimicrobial disks used are each isolates were listed in report. The plates were allowed to stand for at least 30 min before being incubated at 37 °C for 24 h. The tests were in duplicate. After 24 h of incubation, the plates were examined for zones of inhibition. The diameter of the zones of inhibition produced by the antimicrobial were measured and interpreted using the CLSI 2019 zone diameter interpretative standards

7.1.4 ESBL producing E. coli

Discs containing Cephalosporin (Cefotaxime or Ceftriaxone, Ceftazidime, Cefepime) were applied next to a disc with Clavulanic Acid, Amoxicillin + Clavulanic acid or Ticarcillin + Clavulanic Acid. Positive result is indicated when the inhibition zones around any of the Cephalosporin discs are augmented in the direction of the disc containing clavulanic acid.

7.1.5 Modified Hodge test

Modified Hodge Test (MHT) is a simple phenotypic test for detection of presence of Carbapenemase enzyme in bacteria. In short, a 0.5 McFarland dilution of the *E. coli* ATCC 25922 is prepared in 5 ml of broth or saline. A lawn of the 1:10 dilution of *E. coli* ATCC 25922 was streaked onto a Mueller Hinton agar (MHA) plate and allowed to dry 3–5 minutes. A 10 µg Meropenem or Ertapenem susceptibility disk is placed in the center of the test area. In a straight line, test organisms were streaked from the edge of the disk to the edge of the plate. Repeat the same with the QC strain in another direction. The plated are Incubated overnight at 35^{o}C ± 2^{o}C in ambient air for 16–24 hours.

7.1.6 Methicillin resistant Staphylococcus aureus (MRSA)

Cefoxitin disk was used to detect MRSA as per CLSI protocols. Isolates tested against Oxacillin were incubated at 33-35°C (maximum of 35°C) for a full 24 hours before reading.

7.1.7 Vancomycin resistant Enterococci (VRE) detection:

Disk diffusion was used to screen Enterococcal isolates. Those isolates showing phenotypic resistance were screened using PCR for Van A and Van B genes.

7.2 Molecular detection

Standard Polymerase Chain Reaction (PCR) technique was used to detect various resistance genes; ESBL genes (CTX-M, TEM, SHV, OXA, KPC), NDM-1 genes, MecA gene, VanA and VanB. The following primers were used:

TEM F	5'-GCGGAACCCCTATTTG-3'
TEM R	5'-ACC AAT GCT TAA TCA GTG AG-3'
SHV F	5'-TTATCTCCCTGTTAGCCACC-3'
SHV R	5'-GATTTGCTGATTTCGCTCGG-3'
OXA F	5'-GCGTGGTTAAGGATGAACAC-3'
OXA R	5'-CATCAAGTTCAACCCAACCG-3'
KPC F	5'-CGTCTAGTTCTGCTGTCTTG-3'
KPC R	5'-CTTGTCATCCTTGTTAGGCG-3'
NDM F	5'-GGTTTGGCGATCTGGTTTTC-3'
NDM R	5'-CGGAATGGCTCATCACGATC-3'
MEC F	5'-TCCAGATTACAACTTCACCAGG-3'
MEC R	5'-CCACTTCATATCTTGTAACG-3'
Van AF	5'-AAC AAC TTA CGC GGC ACT-3'
Van AR	5'-AAA GTG CGA AAA ACC TTG C-3'
Van B F	5'-GAT ATT CAA AGC TCC GCA GC-3'
Van B R	5'-GGT ATC TTC CGC ATC CAT CA-3'
blaCTXMF	5'-GACGATGTCACTGGCTGAGC-3'
blaCTXMR	5'-AGCCGCCGACGCTAATACA-3'
blaCTXM-3F	5'-CGCTTTGCCATGTGCAGCACC-3'
blaCTXM-3R	5'-GCTCAGTACGATCGAGCC-3'

9. Annex B: Agarose gel electrophoresis for PCR products