Antimicrobial resistance of *Staphylococcus aureus,* fecal streptococci, *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolated from the coastal water of the Gaza strip-Palestine

Elmanama Abdelraouf A.[1], Hartemann Philippe[2], Elnabris Kamal J.[3], Ayesh Adnan[4], Afifi Samir[5], Elfara Fatma[5], Aljubb Alaa R.[5]

1   Medical laboratory Sciences Department, Islamic university-Gaza, P.O Box, 108, Gaza, Palestine
2   Université Nancy Lorraine, Nancy, France
3   Biology Department, Islamic university-Gaza, P.O Box, 108, Gaza, Palestine
4   Geology Department, Al Azhar University- Gaza, P.O. Box 1277, Palestine
5   Earth and environmental Science department, Islamic university-Gaza, P.O Box, 108, Gaza, Palestine

Corresponding author:

Dr. Abdelraouf A. Elmanama

✉   elmanama@iugaza.edu.ps

Abstract

Objectives: To document the occurrence and distribution of antibiotic resistance of clinically important bacteria in the seawater of Gaza strip-Palestine.

Methods: Seawater samples were collected at 16 location distributed along the coast of the Gaza strip. Sampling was accomplished during 12 months, from March 2014 to June 2015. The microbial composition including *Enterobacteriaceae, Staphylococcus aureus,* fecal streptococci and *Pseudomonas aeruginosa* was recorded and tested for their resistance to specific antimicrobial agents according to CLSI using the disc diffusion method.

Results: A total of 816 isolates of *Enterobacteriaceae (*377*), S. aureus (*29*)*, fecal enterococci (FS) (369*)*, and *P. aeruginosa (*29*)* were recovered and identified. *Enterobacteriaceae*, *P. aeruginosa,* FS and *S. aureus* isolates exhibited the highest rates of resistance against β-lactam drugs. The isolates also showed resistance to at least one antimicrobial in the range between 99.7 to 78%. Multiple resistance occurred in almost 85% of all isolates; 99.2% of *Enterobacteriaceae*, 96.6% of *P. aeruginosa*, 72.1% of FS and 61% of *S.aureus*. The incidence of multiple resistance of isolates from all sampling locations ranged from 69.2 to 94.1%. Antibiotic resistance indices were found to be highest in *P. aeruginosa* (0.57), followed by *E. coli* (0.53), FS (0.49), *Enterobacter* (0.41), *S. marcescens* (0.40), *Klebsiella* (0.39) and

finally *Proteus* (0.28). Most of the isolates showed multiple antibiotic resistance (MAR) index value higher than 0.2.

Conclusions: This study demonstrated that the seawater of the Gaza strip is highly contaminated with antibiotic resistant bacteria which can be transmitted to humans through recreational and other activities. Therefore, there is a need to apply appropriate and rationale use of antibiotic to minimize the occurrence of multiple antibiotic resistant bacteria in the marine environment. Proper treatment of sewage before it is discharged to the sea is highly recommended.

Keywords: Multiple antimicrobial resistance, Gaza strip, seawater, fecal enterococci, *P. aeruginosa*, *Enterobacteriaceae*, *S. aureus*.

Introduction

Antimicrobial drugs are used worldwide, not only to treat infectious diseases, but also to act as prophylactic agents to prevent infections, to promote growth in agriculture and aquaculture, and to preserve food [1]. Human intensive and uncontrolled use of antibiotics in medicine, veterinary and agricultural practices have probably led to emergence and spread of antibiotic resistant bacteria in the environments [2].

Generally, bacteria may develop antibiotic resistance by spontaneous chromosomal mutations in the bacterial genome or by acquiring resistance genes through horizontal gene transfer between different bacterial species through conjugation, transformation or transduction [3].

In the Gaza strip, there is growing evidence that the misuse and overuse of antibiotics are the major contributors to the development and spread of antibiotic resistance bacteria in our region. In fact, no regulation exists regarding the use and dispense of antibiotics in the Gaza Strip. Although the problem of drug resistance is not new, the scale of the problem in this region has received little attention. Previous studies of the antibiotic resistance problem have mainly focused on nosocomial infections caused by multidrug resistant pathogens [4-9]. There was only one study, which has investigated the contribution of hospital effluents in spreading of antibiotic resistance in the Gaza's environment [10].

Currently, the pollution of coastal areas represents one of the most difficult challenges because it causes economic damages as well as affects general health of people. Besides that, such pollution may also accelerate the emergence of antibiotic resistance bacteria in marine environment which posing serious threats to humans health and aquatic ecosystems by the increasing the load of antimicrobial resistant bacteria in nature [11].

The effluents of wastewater treatment plants, Wadi Gaza and some sewage outlets from seaside residences, marine fish farms effluents are the main sources of marine pollution with antimicrobial resistance in Gaza strip.

Apart from the adverse effects, several studies suggested that antibiotic resistant bacteria could provide an important indicator of marine water quality and its potential health risk [12-15]. Accordingly, calls have been made to consider testing for antibiotic resistance when establishing bacteriologi-

cal water quality criteria [16]. Recently, the concept of antibiotic resistance was used as a cost effective technique for tracking the source bacterial pollution in the environment [2].

There are no local published studies or reports regarding the occurrence of antimicrobial resistant in marine environments. To our best knowledge, this is the first study aimed to detect the presence of antimicrobial resistance in pathogenic bacteria of seawater samples collected from marine environment in the Gaza strip. The objective of the present study was to document the occurrence and distribution of antimicrobial resistant of clinically important bacteria in the seawater of Gaza strip; and to assess the microbiological safety of coastal water of the Gaza strip.

Materials and Methods

Sampling locations. Seawater samples were collected at 16 locations distributed along the coast of the Gaza strip **(Figure 1)**. Sampling site selection was based on a number of criteria including pre-existing studies and reports, site access and its suitability for sampling and most importantly the different anthropogenic activities to which they are thought to be exposed.

All sampling sites were identified by geographic coordinates as determined by a hand-held Global Positioning System **.** Samples were collected twice a month, over the period from March 2014 to June 2015. A total of 24 samples from each location were collected for analysis. All tests were carried out at the Environmental and Rural Research Center at the Islamic University of Gaza and the Water Institute of Al-Azhar University Gaza–Palestine.

Sample Collection and Processing

Sampling was conducted according to the World Health Organization Manual for Recreational Water and Beach Quality Monitoring and Assessment WHO (1995) [17]. Seawater samples were collected

Figure 1. Location of seawater sampling sites along the coast of Gaza strip.
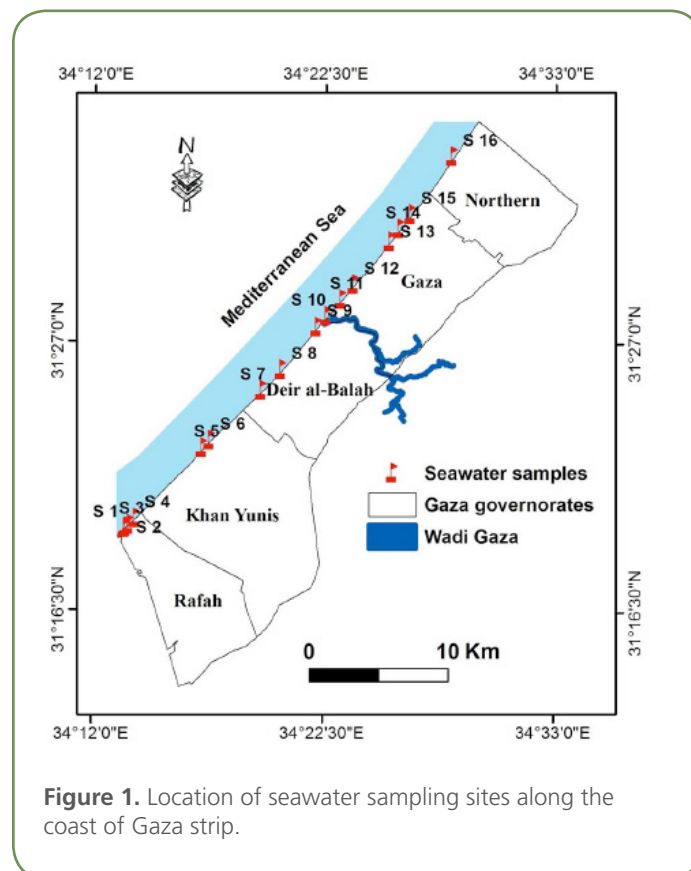
in 2L sterile containers. The bottles were rinsed 2-3 times with seawater from the same site before collection, the lid of the bottle was removed, turned upside down, gently lowered approximately 20-30 cm below the water surface and then turned so that mouth was pointing upward. When the bottle was approximately 2/3 filled, it was lifted above the surface and immediately the lid was placed back on the bottle. All collected samples were kept immediately at 4°C in an icebox and transported to the laboratory for analysis within two hours of collection.

Isolation of Bacteria. Under strict aseptic conditions, appropriate volumes of seawater samples were filtered through 0.45 μm membrane filters. The filters were then placed on M-FC, m-FS, cetrimide agar and Baird-Parker media for growing of fecal coliform (FC), fecal enterococci , *P. eruginosa* and *S. aureus* respectively. The plates were incubated at 44-45 °C for 18-24 h for fecal coliform, at

41±0.5 °C for 48 h for enterococci , at 35 °C for 24 h for *P. aeruginosa* and at 35 ± 0.5 °C for 48 ± 4 h for *S. aureus*. One Blood agar and one MacConkey plates were streaked and incubated at 35 ± 0.5 °C for 24 hours for isolation of potential pathogens.

Bacterial Identification

Preliminary identification of colonies was determined based on morphological characteristics and pigment production on selective media. Various shades of blue colonies on m-FC medium indicated fecal coliform, red colony with a metallic sheen on FS agar indicated streptococci, yellow-green color on Cetrimide agar indicated *Pseudomonas*, smooth and entire colonies with grey to black coloration on Baird-Parker agar indicated Staphylococcus. For the purpose of confirmation, singly growing colonies were picked and purified by re-streaking on fresh media. Pure colonies were then subjected to biochemical tests such as Oxidase, Catalase and API kit (BioMérieux- France) to confirm the identity of the isolated bacteria prior to subsequent antimicrobial susceptibility testing.

Antimicrobial Susceptibility testing

Susceptibility of all isolates were investigated against commercially available antibiotic discs (Himedia) using the standard disk diffusion assay and antimicrobial compounds as guided by Clinical and Laboratory Standards Institute (CLSI). Based on inhibition zone, the isolates were categorized as resistant, intermediate or susceptible to the tested antimicrobials[18].

Multiple Antimicrobial Resistance Index (MARI)

In the current study, MAR index for the isolates was generated for antibiotics as well as for the sites. The MAR index for each isolate was calculated by following the procedure described by Krumperman, P. (1983) using the following formula; number of antimicrobial agents to which the isolate was resistant/ total number of antimicrobials against which isolate was tested[19]. On the other hand, the MAR index for each sample site was calculated as the number of antimicrobials to which all isolates were resistant/ (number of antimicrobials tested × number of isolates tested per site) [20].

Data analysis

Resistance to antimicrobial agents was expressed as percentage. Multiple Antibiotic Resistance index(MARI)for the different isolates and locations was calculated. One way analysis of variance (ANOVA) and multiple comparison testing was performed to determine significant differences among the MAR indices from different sites. Before analysis, all in dices were arcsine transformed to improve normality. Correlations between the geometric means of fecal indicators (fecal coliform and fecal enterococci) and percentage of isolate resistant with respect to different stations were calculated using the Pearson's correlation coefficient. Probabilities <0.05 were considered significant. Statistical analyses in this study were carried out with SPSS(Statistic Package for the Social Science).

Results

Geometric means for the densities of the fecal indicator bacteria, fecal coliform (FC) and fecal enterococci in seawater samples collected from the 16 sites are presented in **Figure 2**. The populations of FS were higher than that of FC at all locations, with geometric means ranged from 248.9-8481.5 for FS and 143.6-3534.3 cfu/100ml for FC. The maximum populations of both FC and FS in seawater samples were observed at S15 with geometric mean values of 3.53×10^3 and 8.48×10^3 cfu/100 ml respectively, followed by S12 (3.48×10^3 and 6.60×10^3), S10 (2.75×10^3 and 3.93×10^3) and S14 (1.74×10^3 and 3.54×10^3).
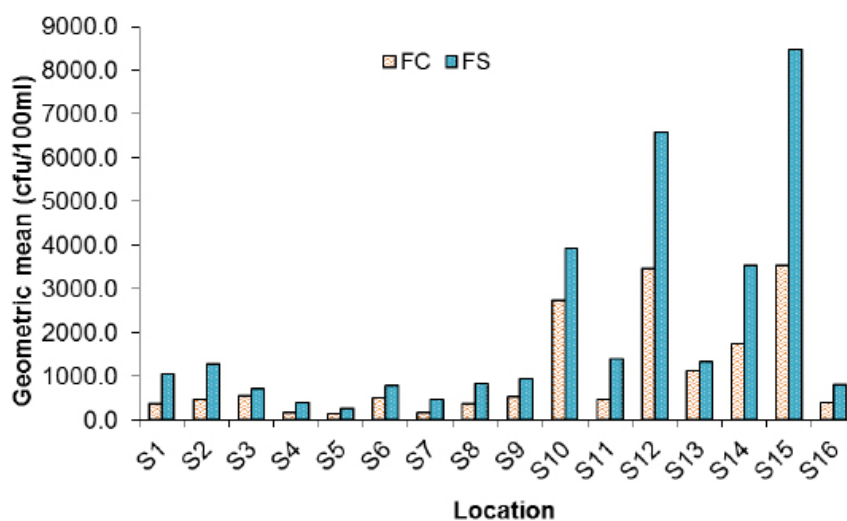
Figure 1. Geometric mean of fecal coliform (FC) and fecal enterococci (FS) in seawater samples at 16 locations.

Bacterial isolates

A total of 816 bacterial isolates were identified to at least the genus level, and examined for antibiotic resistance test by using a selected list of antimicrobial agents for each bacterial group. These isolates (410 gram positive and 406 gram negative) belonging to four groups, including 377 *Enterobacteriaceae*, 369 fecal enterococci , 29 *S. aureus* and 29 isolates were belong to *Pseudomonas*. Enteric bacterial isolates belonged to five genera, with *E. coli* being the most frequent species (272 isolate), followed by 83 isolate of *Klebsiella* spp., 11 of *Enterobacter* spp., 8 of *Serratia marcescens* and 3 of *Proteus vulgaris*.

Resistance profile of bacterial isolates

Table 1 shows the resistance percentage of isolates to different antibiotics. Bacterial groups displayed varying resistance to the tested antimicrobial agents, with Gram-negative bacteria (*Enterobacteriaceae* and *P. eruginosa*) showing higher resistance than Gram-positive bacteria (FE and *S. aureus*). *Enterobacteriaceae* as a group exhibited high rates of resistance against ampicillin and amoxicillin. Amikacin followed by ciprofloxacin and gentamicin were the most effective with resistance rates of 2.1, 26.8 and 28.4% respectively. An overall notable resistance against Imipenem was recorded (57.6%) with 100% resistance of the 3 *Proteus* isolates. *P. aeruginosa* isolates showed phenomenal resistance against the three tested carbapenems; meropnem (86.2%), imipnem (96.6%) and ertapnem 93.1%. amikacin showed the highest activity (only 2.1% resistance).

S. aureus showed high percentage of resistance to β-lactam antibiotics, which include the penicillin G (65.9%) and oxacillin (61%) which are used to detect methicillin resistance. Vancomycin resistance was strikingly high (48.8%). Ciprofloxacin and gentamicin showed the greatest efficacy with only 2.4% resistance, followed by doxycycline and chloramphenicol (4.9% resistance). FS resistance was 16.8% and 39.6% to linezolid and vancomycin respectively. Highest resistance was detected against penicillin G (75.1%) and ampicillin (76.7%).

Table 1. Antibiotic resistance profile of pathogenic bacteria isolated from the seawater samples of all studiedsites (%).

Antimicrobial agent	% resistant								
	Enterobcteriaceae								
	All* (377)**	Escherichia coli (272)	Enterobacter (11)	Klebsiella (83)	P. vulgaris (3)	S. marcescens (8)	S. aureus(41)	P. aeruginosa (29)	Fecal streptocci
Amikacin	2.1	2.2	0.0	2.0	0.0	0.0	NT	3.4	NT
Amoxicillin	97.1	97.8	100	95.2	66.7	100	NT	NT	NT
Ampicillin	98.7	98.9	100	98.8	66.7	100	NT	NT	76.7
Azithromycin	NT	NT	NT	NT	NT	NT	14.6	NT	NT
Aztreonam	30.2	36	18.2	15.7	0.0	12.5	NT	31	NT
Ceftrazidime	40.1	45.6	18.2	25.3	33.3	37.5	NT	NT	NT
Ceftriaxone	NT	NT	NT	NT	NT	NT	NT	34.5	NT
Cefuroxime	71.9	77.6	54.5	55.4	66.7	75.0	NT	NT	NT
Chloramphenicol	36.1	42.6	18.2	19.3	00.0	25.0	4.9	NT	NT
Ciprofloxacin	26.8	31.3	18.2	15.7	00.0	12.5	2.4	37.9	NT
Clindamycin	NT	NT	NT	NT	NT	NT	36.6	NT	NT
Doxycycline	NT	NT	NT	NT	NT	NT	4.9	NT	NT
Ertapenem	NT	NT	NT	NT	NT	NT	NT	93.1	NT
Gentamicin	28.4	29	27.3	30.1	00.0	00.0	2.4	34.5	42.3
Imipenem	57.6	60.7	63.6	45.8	100	50	NT	96.6	NT
Linezolid	NT	NT	NT	NT	NT	NT	NT	NT	16.8
Meropenem	NT	NT	NT	NT	NT	NT	NT	86.2	NT
Ofloxacin	NT	NT	NT	NT	NT	NT	NT	NT	40.1
Oxacillin	NT	NT	NT	NT	NT	NT	61	NT	NT
Penicillin G	NT	NT	NT	NT	NT	NT	65.9	NT	75.1
Piperacillin	50.7	57.4	45.5	33.7	00.0	25	NT	51.7	NT
Rifampin	NT	NT	NT	NT	NT	NT	12.2	NT	NT
Tetracycline	36.9	44.1	18.2	18.1	00.0	25.0	NT	NT	NT
Trimethoprim/Sulfonamides	64.7	67.6	54.5	57.8	33.3	62.5	17.1	NT	NT
Vancomycin	NT	NT	NT	NT	NT	NT	48.8	NT	39.6

* All Enterobacteriaceae isolates tested.
**Values in parentheses indicates the number of isolates examined.
NT: Not tested

The incidence of multidrug resistance

The incidence of multiple resistance, that is, the simultaneous resistance to more than one antimicrobial agent for all isolates from the different sampling locations are listed in **Table 2**. The incidence of multiple resistance occurred in 85% of all isolates and was 99.2% for *Enterobacteriaceae* (99.6% for *E. coli*, 97.6% for *Klebsiella* spp., and 100% for *Enterobacter* spp., *P. vulgaris* and *S. marcescens*), 96.6% for *P. aeruginosa*, 72.1% for FS , and 61% for *S. aureus*. Similarly, the incidence of multiple resistance for all isolates from the 16 sampling locations were ranged from 69.2 to 94.1%, with the highest incidence occurring at S15 and S16 while lowest incidence occurred at S4 and S5.

Table 2. The incidence of multiple resistance of the isolated pathogens from the different sampling sites.

Sampling sites	Enterobacteriaceae						S. aureus	P. aeruginosa	Fecal Enterococci	Total
	All*	E. coli	Klebsiella	Enterobacter	S. marcescens	P. vulgaris				
S1	95.5	93.8	100	N	N	100	100	0.0	61.1	80.0
S2	95.7	100	83.3	N	N	N	50.0	N	70.8	80.4
S3	100	100	100	N	N	N	33.3	100	68.2	80.0
S4	100	100	100	N	N	N	100	N	54.5	73.0
S5	100	100	100	N	N	N	0.0	N	52.4	69.2
S6	100	100	100	100	100	N	100	100	73.1	87.0
S7	100	100	100	N	N	N	N	N	66.7	78.1
S8	100	100	100	N	N	N	50.0	N	66.7	81.6
S9	100	100	100	N	100	N	00.0	100	87.0	92.0
S10	96.9	100	90.9	100	N	N	66.7	100	83.3	90.5
S11	100	100	100	N	100	N	25.0	100	66.7	81.7
S12	100	100	100	N	100	N	100	100	72.0	89.7
S13	100	100	100	100	100	N	100	100	71.4	85.5
S14	100	100	100	N	100	100	50.0	100	81.0	89.3
S15	100	100	100	100	N	N	83.3	100	88.0	94.1
S16	100	100	100	100	100	N	50.0	N	85.7	90.9
Total	99.2	99.6	97.6	100	100	100	61.0	96.6	72.1	84.9

N: Not detected * All Enterobacteriaceae isolates tested.

Multidrug resistance of bacterial isolates

Most of the isolates showed resistance to multiple antibiotics. A prominent proportion of *Enterobacteriaceae* (15.4%) and *S. aureus*(19.5%) isolates exhibited simultaneous resistance against four antimicrobials, whereas FS resistant (21.4%) to one antimicrobial agent followed by two (17.1%), four and five (15.7%) agents (**Table 3**).

The highest resistance (24.1%) among *P. aeruginosa* isolates was observed to three antimicrobial agents. Only small fraction (0.3, 22, 3.4 and 6.5%) of tested *Enterobacteriaceae*, *S. aureus*, *P.aeruginosa* and FS demonstrated susceptibility to the all agents used in the study (13,11,9 and 6, respectively).

On the other hand, 99.7, 78, 96.6 and 78.6% of *Enterobacteriaceae*, *S. aureus*, *P.aeruginosa* and FS isolates respectively showed resistance to at least one antimicrobial. Among *Enterobacteriaceae*; *Enterobacter* spp., *Klebsiella* spp.and *E. coli* showed higher multi-resistance levels than *P. vulgaris* and *S. marcescens*, as they (the former) showed resistance to as many as 11, 12 and 13 antibiotics (out of the 13 tested antimicrobials), while no bacterial resistant to more than 5-8 were observed among the later two species, respectively. Moreover, the data indicates that more than a half (52.6–93.5%) of the tested bacterial strains were resistant to 1–6 antibiotics, whereas minimal percentage (<50%) of these organisms showed resistance to the remaining antibiotics (if any). Except for four isolates (1.5%) of *E. coli* and 32 (8.7%) isolates of FS, no bacteria inhabiting the studied sites were resistant to all tested antibiotics.

Table 3. Percentage of bacterial resistance to antibiotics

No. of antibiotics to which isolate is resistant	% of isolates								
	Enterobacteriaceae								
	All* (377)**	E. coli (272)	Enterobacter (11)	Klebsiella (83)	P. vulgaris (3)	S. marcescens (8)	S. aureus (41)	P. aeruginosa (29)	Fecal streptococci (369)
0	0.3	0.0	0.0	1.2	0.0	0.0	22.0	3.4	6.5
1	0.5	0.4	0.0	1.2	0.0	0.0	17.1	0.0	21.4
2	7.4	5.9	27.3	9.6	33.3	0.0	7.3	6.9	17.1
3	12.7	11.4	0.0	16.9	0.0	37.5	14.6	24.1	14.9
4	15.4	13.2	27.3	20.5	33.3	12.5	19.5	17.2	15.7
5	10.6	9.6	9.1	14.5	33.3	0.0	12.2	17.2	15.7
6	10.6	12.1	9.1	6.0	0.0	12.5	2.4	6.9	8.7
7	6.6	5.5	0.0	10.8	0.0	12.5	0.0	6.9	
8	7.2	6.6	0.0	8.4	0.0	25.0	2.4	17.2	
9	5.8	6.6	9.1	3.6	0.0	0.0	2.4	0.0	
10	7.7	8.8	9.1	4.8	0.0	0.0	0.0		
11	6.6	8.5	9.1	1.2	0.0	0.0	0.0		
12	7.4	9.9	0.0	1.2	0.0	0.0			
13	1.1	1.5	0.0	0.0	0.0	0.0			

* All Enterobacteriaceae isolates tested.
**Values in parentheses indicates the number of isolates examined.

Multiple Antibiotic Resistance Index (MARI)

The MAR index values for isolated strains from the different locations and the percentage of MAR index value >0.2 are given in **Table 4**. The overall MARI value for all isolates (n = 816) from all locations was found to be 0.48 and 81% of these isolates have MARI values higher than 0.2. The MARI value for isolates were ranged from an average of 0.25 (*S. aureus*) to 0.57 (*P. aeruginosa*). The MAR index values for other strains were as follows; 0.53 for *E. coli*, 0.49 for FS , 0.41 for FS, 0.40 for *S. marcescens*, 0.39 for *Klebsiella* and 0.28 for *Proteus*. Among

theisolates, 92% of *Enterobacteriaceae*, 72% of FS, 54% of *S. aureus* and 97% of *P. aeruginosa* had a high MAR index value (>0.2).

For all the sites, the percentage of MARI > 0.2 ranged from 61% (S5) to 91% (S15). The highest antimicrobial resistance was detected among the bacteria isolated from S15 (MARI 0.61), followed by S10 (MARI 0.57), S12 (MARI 0.55), S11 (MARI 0.52), S2, S9, S14 (MARI 0.49), S13 (MARI 0.47), S8 (MARI 0.46), S16 (MARI 0.45), S6 (MARI 0.44), S3 (MARI 0.43), S1, S7 (MARI 0.41) and finally S4 and S5 with MARI value of 0.33.

Despite the observed differences among the

Table 4. Multiple antibiotic resistance index of the isolated strains from different stations (%) and the percentage of MAR index value >0.2

Sampling sites	Enterobacteriaceae						Fecal Streptococci	S. aureus	P. aeruginosa	Total	Isolates with MARI value > 0.2 (%)
	All*	E. coli	Klebsiella	Enterobacter	S. marcescens	Proteus spp					
S1	0.41	0.46	0.28	N	N	0.15	0.46	0.03	0.0	0.41	71
S2	0.52	0.57	0.41	N	N	N	0.51	0.20	N	0.49	78
S3	0.51	0.52	0.42	N	N	N	0.40	0.12	0.56	0.43	80
S4	0.31	0.29	0.38	N	N	N	0.34	0.27	N	0.33	68
S5	0.36	0.35	0.38	N	N	N	0.33	0.05	N	0.33	61
S6	0.48	0.54	0.27	0.31	0.31	N	0.42	0.18	0.22	0.44	83
S7	0.34	0.30	0.50	N	N	N	0.44	N	N	0.41	78
S8	0.49	0.50	0.42	N	N	N	0.46	0.18	N	0.46	80
S9	0.43	0.47	0.28	N	0.23	N	0.55	0.00	0.89	0.49	90
S10	0.59	0.72	0.38	0.56	N	N	0.59	0.30	0.53	0.57	89
S11	0.54	0.55	0.49	N	0.62	N	0.53	0.11	0.61	0.52	82
S12	0.60	0.70	0.42	N	0.54	N	0.52	0.45	0.47	0.55	82
S13	0.46	0.51	0.44	0.23	0.23	N	0.46	0.36	0.78	0.47	80
S14	0.48	0.55	0.34	N	0.54	0.35	0.57	0.23	0.39	0.49	87
S15	0.63	0.73	0.55	0.44	N	N	0.68	0.33	0.44	0.61	91
S16	0.37	0.44	0.26	0.31	0.23	N	0.52	0.45	N	0.45	79
Total	0.49	0.53	0.39	0.41	0.40	0.28	0.49	0.25	0.52	0.48	81
MARI value > 0.2 (%)	92	94	88	73	100	67	72	54	97	81	

N: Not detected   * All Enterobacteriaceae isolates tested.

MAR indices of isolates from different locations, it was only the *Enterobacteriaceae* (especially *E. coli*) and FS isolates that showed significant differences (ANOVA, p < 0.05) between the MARI values at some locations. This is especially true for S10, S12 and S15 versus S4, S5 and S7.

Correlation analysis between the percentage of resistance and the density of fecal indicators at the same location showed a strong, positive and significance (*p* < 0.001) correlation with correlation coefficients (*r*) of 0.79 and 0.78 for FC and FS respectively. Furthermore, seawater samples from locations such as Alshatee refugee camp (S15), Nitsareem cross (S12), Wadi Gaza (S10) and the basin of Gaza fishing port (S14) showed the highest density of fecal indicators. At the same time, isolates from these locations exhibited the highest resistance. On the other hand, isolates from samples collected at locations such as S4 (Remas restaurant), S5 (Al zaeem restaurant) and S7 (Sonista restaurant) from Kan-Younis and Middle governorates showed lower levels of resistance and as expected, these locations showed lowest density of fecal indicators.

Discussion

Microbiological examination of marine samples for pathogenic bacteria is of great importance, representing a direct measurement of deleterious effect of microbiological pollution. Present investigation demonstrated higher densities of FS than that of FC in all locations. Such variation was consistent with the findings of Dufour A. (1977) [21] and may be attributed to the different rates of growth and survival of these microorganisms in marine environment [22] and to the differences in susceptibility to physical factor, such as exposures to varying level of sunlight intensities [23]. The findings that FC and FS populations in seawater are increased toward the north **(Figure 2)** could be attributed to the natural coastal processes dominating the Palestinian coastline such as waves regimes and wave generated long shore current which are responsible for generating net longshore transport to the north, thus, carrying more pollutants toward the northern locations.

The results of this study provide clear evidence of the considerable spread of multiple antimicrobial resistance among four groups of clinically important bacteria namely; *Enterobacteriaceae*, FS, *P. aeruginosa* and *S. aureus* isolates in the seawater samples from the different locations at Gaza strip. The occurrence of bacteria in aquatic environment with high resistance to antimicrobial may be an indication of the widespread and prolonged misuse of these antibacterial drugs in the Gaza strip [8].

The high value of resistance found among *Enterobacteriaceae* genera to ampicillin (98.7%), amoxicillin (97.1%) and cefuroxime (71.9%) which all belong to β-Lactam was in agreement with that reported byAbo-State *et al* (2012) [24] and DebMandal *et al* (2011) [25].

Fecal isolates possessed maximum resistance to ampicillin (76.7%) and penicillin G (75.1%). Entertococci resistance to ampicillin is of great importance, since ampicillin is used in treatment of such infections [26]. Various rates of ampicillin resistance in both clinical and environmental isolates of FS isolates were previously reported from the Gaza strip [4, 10].

In the present study, *P. aeruginosa* isolates from marine environment demonstrated high resistance rate (> 50%), with remarkable high resistance to imipenem (96.6%), ertapenem (93.1%), meropenem (86.2%) and piperacillin (51.7). This high rate of resistance could be attributed to extended spectrum B-lactamase (ESBL) and metallo B-lactamase (MBL) encoding genes [27].

S. aureus showed high level of methicillin resistance as indicated by their resistance to oxacillin (61%). Previously, Abu Hujier and Sharif (2008) had reported prevalence of Methicillin-resistant *S. aureus* (MRSA) of 22% from clinical isolates obtained

from patients in Gaza strip [28]. More recently however, Al Laham (2012) reported MRSA prevalence of 62.1% in operation theaters at main hospitals of Gaza strip[5]. Previous studies from marine environments from other localities around the world found much lower percentages than the current study [29, 30]. The observed higher rate of resistance among *S. aureus* isolates in this study however, may be due to the inter-generic transfer of resistance among different genera of Gram-positive cocci and between *Bacillus* species and Staphylococci and Streptococci which may increase in aquatic environment[31].

The results of this study indicated that isolates belonging to Gram-negative bacteria (*Enterobacteriaceae* and *P. aeruginosa*) exhibited higher resistance than Gram-positive bacteria (FS and *S. aureus*), although the difference is not statistically significant (P = 0.085, independent samples t-test). The predominance of antimicrobial resistance among Gram-negative bacteria has been reported previously [32].

This study revealed the occurrence of multidrug resistant (MDR) bacteria in coastal water of the Gaza strip. The varying frequencies of antimicrobial resistance found in the present study was generally consistent with those of other studies toward bacteria from different natural environments including seawater [33-35].

In the current study, 81% of the total isolates, 91.8% of *Enterobacteriaceae*, 72.1% of FS, 96.6% of *P. eruginosa* and 53.7% of *S. aureus* showed MAR index of more than 0.2 and up to 1 indicating very high degree of resistance . Calculation of MARI values of the sites revealed that all (the 16 locations) had a high overall MAR index value (> 0.2) and consequently, represent a high risk source of fecal pollution.

A comparison between fecal indicators and the percentage of resistance at the same location in this study showed a clear trend; i.e., as the density of fecal indicators increases, so did the percentage of resistant isolates. The findings of strong correlation between the densities of fecal indicators and the percentage of resistant isolates agrees with previous studies conducted in different localities around the world which showed positive correlations between the degree of pollution and bacterial resistance to antibiotics[12, 35].

The widespread of antibiotic resistant bacteria and fecal indicators in the shore of Alshatee refugee camp (S15) might due to the release of untreated wastewater into this region. In addition, (S12) location is exposed to continuous discharge from a nearby fish farm (possible source of antimicrobials and antimicrobial resistant pathogens).

The obtained results confirmed the presence of the multidrug resistance bacteria in the coastal water of Gaza strip. The prime source of antimicrobials in this area may be the hospitals; aquaculture and agriculture were antimicrobial are widely used as therapy for bacterial infections in humans and in animals as growth promoters . In addition, the effluents of Wadi Gaza, which mainly consist of untreated sewage, are discharged directly into the shore. Several studies reported that antibiotic resistance genes could be transferred in aquatic environments among pathogenic and nonpathogenic bacteria, making it a reservoir for resistance genes and thus, representing a high environmental risk for population[2, 36].

Conclusions

The findings in this study demonstrated the occurrence of high rates of multiple antibiotic resistance among potential pathogens isolated from seawater samples along the Gaza strip seashore. Vancomycin-resistant enterococci , MRSA, carbapenems-resistant *P. eruginosa* and MDR *Enterobacteriaceae* were recovered. This condition requires immediate actions of the concerned authorities to prevent untreated domestic and hospital wastewater from being discharged into seawater.

Acknowledgment

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