# Contribution of Hospital Wastewater to the Spread of Antibiotic Resistance in Comparison to Non-Health Institution

**Dr. Abdelraouf A. Elmanama** *
**Dr. Abboud Y. ElKichaoui** **
**Miss. Mai Mohsin** ***

## الملخص

الزيادة العامية لمشكلة المقاومة للمضادات الحيوية بالإضافة للانحدار في اتجـاه تطـوير مضادات حيوية جديدة له مضاعفات خطيرة. لقد صمم هذا البحث لدراسة صور مقاومة البكتيريا للمضادات الحيوية ولقد تم عزل هذه البكتيريا من مستشفى الشفاء في قطاع غزة كمؤسسة صحية ومقارنة صور المقاومة هذه مع بكتيريا عزلت من مؤسسات غير صحية. في هذه الدراسة جمعت عينات المياه العادمة من ثلاث أماكن مختلفة من مستشفى الشفاء وثلاث أماكن مختلفة من الجامعة الإسلامية في قطاع غزة ومن مدخل ومخرج محطة غزة لمعالجة المياه العادمة وعينة من ميـاه البحر بالقرب من مصب محطة المعالجة في البحر . مجموع العينات التي تم جمعهـا كـان 45 عينة وقد تم عزل 154 بكتيريا مختلفة من هذه العينات. وكانت نسب كل من البكتيريا المعزولة كالتالي:

30.5% *E. coli*, 33.1% *Pseudomonas* spp., 10.4% *Klebsiella* spp., 4.5% *Proteus* spp. and 21.4% *Enterococcus* spp.

هذه البكتيريا اجري لها اختبار الحساسية للمضادات الحيوية. وكانت نسبة مقاومة البكتيريا السالبة لخمسة عشر مضادا حيويا على النحو التالي:

Cephalexin (52.1%), Co-Trimoxazole (41.3%), Tetracycline (41.3%), Chloramphenicol (39.7%), Nalidixic Acid (36.4%), Piperacillin (28.9%), Amoxycillin (35.5%), Ceftizoxime (14.0%), Azreonam (13.2%), Ciprofloxacin (12.4%), Tobramycin (11.6%), Gentamicin (10.7%), Ceftazidime and Amikacin (8.3%) and Imipenem (0.0%).

ونسبة مقاومة البكتيريا الموجبة لخمس مضادات حيوية على النحو التالي:

* Medical Technology Department, Faculty of Science, Islamic University of Gaza, Palestine, elmanama_144@yahoo.com
** Biology Department, Faculty of Science, Islamic University of Gaza, Palestine
*** Medical Technology Department, Faculty of Science, Islamic University-Gaza. Palestine.

Streptomycin (91.0%), Vancomycin (75.8%), Erythromycin (60.6%), Teicoplanin (9.1%) and Ampicillin (6.1%).

ولقد وجد أن البكتيريا المعزولة من عينات المياه العادمة الخارجة من مستـشفى الـشفاء ومن مبنى المختبرات في الجامعة الإسلامية تحتوي على عدد اكبـر مـن البكتيريـا المقاومـة للمضادات الحيوية بالمقارنة مع الأماكن الأخرى.

## ABSTRACT

A potential post-antibiotic era is threatening present and future medical advances. The current worldwide increase in resistant bacteria and, simultaneously, the downward trend in the development of new antibiotics have serious implications. This research conducted to study the resistance profile of bacterial isolates from Al-Shifa hospital in Gaza as a health institution and comparing their profile to a non-health institution. In this study, wastewater sample were collected from three different sewers receiving wastewater in Al-Shifa hospital, from three sewers receiving wastewater in Islamic University of Gaza (IUG), from inlet and outlet of Gaza wastewater treatment plant (WWTP) and from sea water. A total of 45 samples were collected and 154 different bacteria were isolated from these samples. From the isolated bacteria 30.5% were *E. coli*, 33.1% *Pseudomonas* sp., 10.4% *Klebsiella* sp., 4.5% *Proteus* sp. and 21.4% *Enterococcus* sp. Isolates were subjected to antimicrobial susceptibility testing. the percent of resistance for Gram-negative bacteria to 15 antibiotics were as the following Cephalexin (52.1%), Co-Trimoxazole (41.3%), Tetracycline (41.3%), Chloramphenicol (39.7%), Nalidixic Acid (36.4%), Piperacillin (28.9%), Amoxycillin (35.5%), Ceftizoxime (14.0%), Azreonam (13.2%), Ciprofloxacin (12.4%), Tobramycin (11.6%), Gentamicin (10.7%), Ceftazidime and Amikacin (8.3%) and Imipenem (0.0%). The percent of resistance for Gram-positive bacteria (*Enterococcus*) to 5 antibiotics were as the following: Streptomycin (91.0%), Vancomycin (75.8%), Erythromycin (60.6%), Teicoplanin (9.1%) and Ampicillin (6.1%)

**Keywords**: Antimicrobial resistance, hospitals wastewater, Gaza**.**

## INTRODUCTION:

Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today **(Stuart, 2002).** Confronted by increasing amounts of antibiotics over the past 60 years, bacteria have responded to the deluge with the propagation of progeny no longer susceptible to them. While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria also contributes to the problem **(Stuart, 2002).**

Antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms inhabiting terrestrial and aquatic habitats. However, higher numbers of resistant bacteria occur in polluted habitats compared with unpolluted habitats, indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment **(Baya et al., 1986 and Pathak, 1993)**.

The emergence and spread of antimicrobial resistance are complex problems driven by numerous interconnected factors **(WHO, 2002).** The widespread and often inappropriate administration of antibiotics in livestock, pets, and humans has been shown to result in the development of antibiotic-resistant bacteria and is generally accepted to be the primary pathway for proliferation of antibiotic-resistant bacteria in the environment **(Wegener et al., 1999).**

Possible mechanisms by which humans enhance the spread of antibiotic resistance among environmental bacteria include the deliberate or accidental introduction of antibiotics, resistant bacteria and resistance genes into the environment. Antibiotics exert a selection in favor of resistant bacteria by killing or inhibiting growth of susceptible bacteria; resistant bacteria can adapt to environmental conditions and serve as vectors for the spread of antibiotic resistance **(Wegener et al., 1999 and Kruse, 1999).**

The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogen (**Wegener et al., 1999 and Kruse, 1999).**

There are several routes of entry of antimicrobial agents into the environment. Studies have shown that introduction by these routes has changed the antibiotic susceptibility of the microbes in those environments **(Chitnis et al., 2000).**

One of these routes is the sewage, the antibiotics that we take in are not all processed by our bodies. Some of them are expelled as waste and wind up in our wastewater treatment plants. Of bacteria isolated from sludge remaining

after wastewater treatment at one plant, 46.4% were resistant to multiple antibiotics. Sewage from hospitals and pharmaceutical plants has been shown to contribute to antibiotic resistance in treatment plants **(Chitnis et al., 2000).**

The volume of antibiotics used in hospitals and private households and released into effluent and municipal sewage indicates a selection pressure on bacteria **(Kümmerer and Henninger, 2003).** Waste effluent from hospitals contains high numbers of resistant bacteria and antibiotic residues at concentrations able to inhibit the growth of susceptible bacteria **(Grabow & Prozesky, 1973, Linton, 1974, Fontaine and Hoadley, 1976).** Accordingly, hospital waste effluent could increase the numbers of resistant bacteria in the recipient sewers by both mechanisms of introduction and selection for resistant bacteria **(Al-Ahmed et al., 1999).**

Due to heavy antibiotic use, hospital wastewater contains larger numbers of resistant organisms than domestic wastewater. In Florida, vancomycin resistant *E. faecium* were isolated, without enrichment, from hospital wastewater **(Harwood et al., 2002).** VRE were found in 35% of the hospital sewage samples in two Swedish studies **(Blanch, 2003 and Iversen et al., 2002)**. Twenty-five percent of enterococci were vancomycin resistant in a German study of biofilms from hospital wastewater, and of these, many were multiply resistant **(Schwart et al., 2003). Reinthaler et al. (2003)** showed significantly higher percentages of *E. coli* in the inlet water of a treatment plant receiving hospital waste than two other treatment plants. A study of Acinetobacter showed that an increase in the prevalence of oxytetracycline resistance was correlated with hospital wastewater **(Guardabassi et al., 1998).**

Although sewage treatment processes reduce the numbers of bacteria in wastewater, the effluent will still generally contain large numbers of both resistant and susceptible bacteria. **Schwartz et al. (2003)** showed a decrease in VRE from 16% in untreated wastewater to 12.5% at the outlet. High numbers of resistant coliforms have also been found in treatment plant effluents **(Reinthaler et al., 2003)** and rivers receiving effluent from treatment plants have higher numbers of resistant organisms.

In Gaza there are no studies or data concerning resistance profiles of microorganisms isolated from hospital or community sewage. This study was an attempt to generate original local data and examine the possibility of hospital effluents contributing to the resistance problem .

# MATERIALS AND METHODS:

## Sample Site Selection

As part of the study samples of sewage were collected from sewers and sewage treatment plants. Four different sites were selected for the study and from each site samples were collected from different locations. Table (1) illustrates the sampling sites and locations.

**Table 1**. **Sampling sites and locations**

| Sites | Location of sample collection at each sites |
|---|---|
| **Al-Shifa Hospital** | Intensive care unit sewer, Burn unit sewer and Laboratories sewer |
| **IUG** | L building sewer, M building sewer and Laboratories building sewer |
| **GWWTP** | From inlet and outlet |
| **Seawater** | About 20 meters from the point of sewage discharge in seawater |

## Sample Collection:

Wastewater and seawater samples were collected at approximately 2-weeks intervals over a period of 3 months from each of 9 locations. From each site the samples were collected five times. From each location, at each sampling visit, one wastewater sample was taken. Samples were transported in ice box to the laboratory and processed within 2 hours of collection.

## Sample Processing:

Each sample was inoculated on blood agar, MacConkey agar, M-Enterococcus Agar, Pseudomonas Agar, HiCrome UTI Agar plates and Brain heart infusion broth tubes using bacteriological loop and incubated aerobically at 37°C for 24–48 hours. Growing colonies were identified biochemically in a systematic way according to standard methods **(Vandepitte et al., 1996).**

All Gram-negative rods were identified by using API 20E strips. The initial characterization of enterococci was based on catalase reaction, hemolysis, and colony morphology. Further identification of enterococci was accomplished by the use of bile esculin test.

## Antimicrobial Susceptibility Testing:

Bacterial susceptibility testing was done by the disk diffusion method according to Kirby-Bauer method (Bauer et al., 1966) following the NCCLS assessment criteria **(NCCLS, 2001).** Bacterial inocula were prepared by suspending the freshly grown bacteria in 4-5 ml sterile BHIB and the turbidity was adjusted to that of a 0.5 McFarland standard. The inoculum suspension was spread in three directions on a Mueller Hinton agar plate surface with a sterile swab Filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (HiMedia)

The antimicrobial disks tested for all isolates were: Amikacin 30µg, Amoxycillin 30µg, Aztreonam 30µg, Ceftazidime 30µg, Ceftizoxime 30µg, Cephoxitin 30µg, Chloramphenicol 30µg, Ciprofloxacin 30µg, Co-Trimoxazole 23.75µg, Gentamicin 10µg, Imipenem 10µg, Nalidixic Acid 30µg, Piperacillin 100µg, Tetracycline 30µg, Tobramycin 10µg, were tested against Gram negative bacteria. On the other hands, Streptomycin 10µg, Tetracycline 30µg, Teicoplanin 30µg, Vancomycin 30µg, Ampicillin 10µg, Erythromycin 15µg were tested against Gram positive bacteria.

The plates were incubated aerobically at 37°C for 18-24 hours. Zone of inhibition around antibiotic disks were recorded and using the chart provided by the antimicrobials manufacturer, results were interpreted as sensitive, intermediate or resistant.

## RESULTS:

This study was conducted during the period of June, 2005 to September, 2005, and attempted to isolate *E. coli*, *Pseudomonas* sp., *Proteus* sp., *Klebsiella* sp., and *Enteroccocus* sp. from wastewater sample for the purpose of studying the possible contribution of Al-Shifa hospital to the increasing problem of antibiotic resistance. Standard antimicrobial susceptibility test was performed for all isolates.

A total of 45 wastewater samples were collected from 9 different sampling points. Each point was sampled 5 times with 2 weeks intervals. Three sampling points at Al-Shifa hospital (burn unit sewer, ICU unit sewer and laboratory sewer), another three from Islamic University-Gaza (L building sewer, M building sewer and laboratory building sewer), two from the Gaza Wastewater Treatment Plant (Inlet and outlet of the plant) and one from the seawater near the GWWTP discharge point. From the 45 wastewater

samples, 154 bacterial strains were isolated. The highest number of bacteria isolated from Al-Shifa hospital and accounted for 32.5% (50 isolates) of the isolated bacteria followed by sites from the Islamic University-Gaza, 29.9% (46), GWWTP, 26.6% (41) and seawater 11.0% (17), as illustrated in details in Table (2) .

**Table 2. Number of bacteria isolated from each sampling point**

| Site | Bacteria | | | | | |
|---|---|---|---|---|---|---|
| | E. coli | Pseudomonas | Klebsiella | Proteus | Enterococcus | Total |
| Burn | 6 | 6 | - | 1 | 5 | 18 |
| ICU | 6 | 4 | 2 | 1 | 3 | 16 |
| Laboratory | 3 | 7 | 2 | - | 4 | 16 |
| L Building | 6 | 4 | 2 | - | 1 | 13 |
| M building | 5 | 6 | 2 | - | 5 | 18 |
| Lab. U | 5 | 5 | 2 | - | 3 | 15 |
| Inlet | 5 | 7 | 1 | 3 | 7 | 21 |
| Outlet | 7 | 7 | 3 | 1 | 2 | 20 |
| Sea | 6 | 5 | 2 | 1 | 3 | 17 |
| Total | 47 | 51 | 16 | 7 | 33 | 154 |

The most frequently identified bacterium was *Pseudomonas* sp. (33.1%) followed by *E. coli* (30.5%), *Enterococcus* sp. (21.4%), *Klebsiella* sp. (10.4%) and *Proteus* sp. (4.5%).

The Gram-negative isolates showed wide variation in their response to the tested antimicrobial drugs as shown in Table (3). High resistance rate to amoxicillin (43.1%), ceftizoxime (33.3%), nalidixic acid (72.5%), cephalexin (90.2%), aztreonam (19.6%), and ceftazidime (13.7%) was observed among *Pseudomonas* sp. The highest resistance rate for tetracycline (100%), amikacin (100%), chloramphenicol and co-Trimoxazole (71.4%) was exhibited by *Proteus* sp. For imipenem antibiotic, there was no resistance at all.

**Table 3. Resistance rates (%) of Gram-negative to antibiotics**

| Antimicrobial | E. coli | Pseudomonas | Klebsiella | Proteus |
|---|---|---|---|---|
| Imipenem | 0 | 0 | 0 | 0 |
| Tetracycline | 31.9 | 49 | 18.8 | 100 |
| Amikacin | 10.6 | 3.9 | 18.8 | 100 |
| Amoxycillin | 36.2 | 43.1 | 25 | 0 |
| Tobramycin | 12.8 | 11.8 | 12.5 | 0 |
| Ceftizoxime | 0 | 33.3 | 0 | 0 |
| Nalidixic Acid | 14.9 | 72.5 | 0 | 0 |
| Cephalexin | 31.9 | 90.2 | 12.5 | 0 |
| Co-Trimoxazole | 29.8 | 58.8 | 6.3 | 71.4 |

| | | | | |
|---|---|---|---|---|
| Piperacillin | 42.6 | 17.6 | 31.3 | 14.3 |
| Ciprofloxacin | 14.9 | 13.7 | 0 | 14.3 |
| Aztreonam | 10.6 | 19.6 | 6.3 | 0 |
| Chloramphenicol | 12.8 | 70.6 | 6.3 | 71.4 |
| Gentamicin | 10.6 | 7.3 | 25 | 0 |
| Ceftazidime | 6.4 | 13.7 | 0 | 0 |

In Vitro activities of 15 different antibiotics against the Gram-negative bacterial isolates are illustrated in Table (4). A high resistance rate among Gram-negative was observed against cephalexin (52.1%) followed by Co-Trimoxazole and tetracycline (41.3%), chloramphenicol (39.7%), nalidixic acid (36.4%) and amoxicillin (35.5%). The lowest resistance was to amikacin and ceftazidime (8.3% )

**Table 4. Antibiogram of 15 different antibiotics against the Gram-negative isolates**

| Antibiotics | Susceptibility Percentage | | |
|---|---|---|---|
| | Resistance | Intermediate | Sensitive |
| Amoxycillin | 35.5 | 18.2 | 46.2 |
| Amikacin | 8.3 | 19.8 | 71.9 |
| Azreonam | 13.2 | 34.7 | 52.1 |
| Cephalexin | 52.1 | 38.0 | 9.9 |
| Co-Trimoxazole | 41.3 | 9.1 | 49.6 |
| Chloramphenicol | 39.7 | 4.1 | 56.2 |
| Ciprofloxacin | 12.4 | 11.6 | 76.0 |
| Ceftazidime | 8.3 | 8.3 | 83.5 |
| Ceftizoxime | 14.0 | 16.5 | 69.4 |
| Gentamicin | 10.7 | 8.3 | 81.0 |
| Imipenem | 0.0 | 0.0 | 100 |
| Nalidixic Acid | 36.4 | 12.4 | 51.2 |
| Piperacillin | 28.9 | 28.1 | 43.0 |
| Tetracycline | 41.3 | 26.4 | 32.2 |
| Tobramycin | 11.6 | 7.4 | 81.0 |

The resistance pattern for each bacterium varied according to the site from which the bacteria were isolated. For *E. coli* the highest resistance rate to tetracycline, amoxicillin, ciprofloxacin and chloramphenicol was for those isolated from the inlet of the GWWTP and were (66.7%), (66.7%), (66.7%) and (33.3%) respectively. For tobramycin, nalidixic acid, cephalexin, Co-Trimoxazole, piperacillin, gentamicin and Ceftazidime the highest rate of resistance for *E. coli* which was isolated from the laboratory building of

IUG and account for (40.0%), (40.0%), (80.0%), (80.0%), (80.0%), (40.0%) and (40.0%) respectively.

The only Gram-positive isolate was *Enteroccocus* sp. and showed the highest rate of resistance to streptomycin (91.0%). Also it was, 75.8%, 60.6%, 39.3%, 9.1% and 6.1%, for vancomycin, terythromycin, tetracycline, teicoplanin and ampicillin, respectively. This high resistance rate observed for all *Enterococcus* spp., regardless of the isolation site is shown in Table(5)

**Table 5. Percentage resistance of *Enterococcus* sp. isolated from different site to antibiotics**

| Site | No. of isolates | A | Te | S | E | Va | T |
|---|---|---|---|---|---|---|---|
| Burn | 5 | 0.0 | 0.0 | 100 | 60.0 | 80.0 | 60.0 |
| ICU | 3 | 0.0 | 0.0 | 100 | 50.0 | 100 | 0.0 |
| Laboratory | 4 | 0.0 | 28.2 | 85.7 | 100 | 85.7 | 57.1 |
| L building | 1 | 33.3 | 0.0 | 100 | 33.3 | 100 | 33.3 |
| M building | 5 | 0.0 | 0.0 | 100 | 100 | 0.0 | 0.0 |
| Lab. B | 3 | 25.0 | 0.0 | 100 | 100 | 100 | 50.0 |
| Inlet | 7 | 0.0 | 0.0 | 66.7 | 33.3 | 33.3 | 33.3 |
| Outlet | 2 | 0.0 | 0.0 | 100 | 20.0 | 60.0 | 40.0 |
| Sea | 3 | 0.0 | 33.3 | 66.7 | 33.3 | 66.7 | 0.0 |

A: Ampicillin, Te: Tecoplanin , S: Streptomycin, E: Erythromycin,
Va: Vancomycin and T: Tetracycline

A high proportion of the isolated strains showed resistance to more than two drugs. The multiple drug resistance of the isolates is illustrated in Table (6)

**Table 6. Multiple resistance patterns of the isolated strains**.

| Resistance | *E. coli* N=47 | | *Pseudomonas* sp. N=51 | | *Enterococcus* sp. N=33 | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Not resistant | 11 | 23.4 | 2 | 3.9 | 1 | 3.00 |
| To one antibiotic | 10 | 21.3 | 2 | 3.9 | 3 | 9.1 |
| To two antibiotics | 5 | 10.6 | 4 | 7.8 | 11 | 33.3 |
| To three antibiotics | 6 | 12.8 | 8 | 15.7 | 9 | 27.3 |
| To four antibiotics | 6 | 12.8 | 8 | 15.7 | 8 | 24.2 |
| More than 4 | 9 | 19.1 | 27 | 52.9 | 1 | 3.0 |

## DISCUSSION:

This study was conducted to study the contribution of wastewater effluent from different parts of Al-Shifa hospital on the prevalence of resistant bacteria in the recipient sewers in comparative with the contribution of wastewater effluent from non-health institution (Islamic university of Gaza and Gaza wastewater treatment Plant) on the prevalence of resistant bacteria, and to see the impact of wastewater effluent from GWWTP in the seawater.

In this study, different aspects concerning the occurrence and fate of antibiotic resistant bacteria in sewage was investigated:

- The effects of waste effluent from a hospital on the prevalence of resistant bacteria in the recipient sewers.
- The susceptibility pattern of bacteria isolated from wastewater effluent from IUG and GWWTP.
- The impact of wastewater effluent from GWWTP in the prevalence of resistant bacteria in the recipient seawater.

From the total of 45 wastewater and seawater samples that were collected from 9 different sampling points, 154 bacterial strains were isolated. The most frequently identified bacterium was *Pseudomonas* sp. (33.1%) followed by *E. coli* (30.5%), *Enterococcus* sp. (21.4%), *Klebsiella* sp. (10.4%) and *Proteus* sp. (4.5%).

Our results indicate that high incidence of antibiotic resistance among both gram-negative and gram-positive isolates. For gram-negative bacteria, high resistance rate to amoxicillin (43.1%), ceftizoxime (33.3%), nalidixic acid (72.5%), cephalexin (90.2%), aztreonam (19.6%), and ceftazidime (13.7%) was observed among *Pseudomonas* sp. The highest resistance rate for tetracycline (100%), amikacin (100%), chloramphenicol and co-Trimoxazole (71.4%) was exhibited by *Proteus* sp. For imipenem antibiotic, there was no resistance at all. For gram-positive isolate (*Enterococcus* sp.) ,The highest resistance rate was to streptomycin (91.0%). Also it was, 75.8%, 60.6%, 39.3%, 9.1% and 6.1%, to vancomycin, terythromycin, tetracycline, teicoplanin and ampicillin, respectively. The lowest resistance was to ampicillin (6.1%).

In comparison with the levels of antibiotic resistance reported in the literature for clinical isolates **(Astal et al., 2002),** *Pseudomonas* spp. isolates from sewage were generally more susceptible to antimicrobial agents .

The resistance pattern for each bacterium varied according to the site from which the bacteria were isolated. For *E. coli* the highest resistance rate to

tetracycline, amoxicillin, ciprofloxacin and chloramphenicol was for those isolated from the inlet of the GWWTP, the same result was reported in previous study and indicated that the highest resistance rates were found in *E. c*oli strains of a sewage treatment plant which treats not only municipal sewage but also sewage from a hospitals **(Reinthaler et al., 2003).** For tobramycin, nalidixic acid, cephalexin, Co-Trimoxazole, piperacillin, gentamicin and Ceftazidime the highest rate of resistance for *E. coli* which was isolated from the laboratory building of IUG .

With regard to *Pseudomonas* sp., the resistance rate is shown to be high for most antibiotics and reached 100% in some sites for nalidixic acid, cephalexin, Co-Trimoxazole and chloramphenicol. The *Pseudomonas* sp. strains isolated from Al-Shifa hospital and sea were more resistant to antibiotics than *Pseudomonas* sp. isolated from other sites.

The most resistance *Klebsiella* sp. isolate was that isolated from the laboratory building of the IUG. The highest resistance rate of *Klebsiella* was observed against piperacillin (100%). One explanation to this high resistance rate for the bacteria that isolated from the laboratory of the IUG may be due to heavy metals, biocides and various chemicals that discharged in sewage of this building and these substances have the potential to select for antibiotic resistance **(Foster, 1983).**

*Enteroccocus* sp. showed highest rate of resistance to streptomycin followed by vancomycin and erythromycin. This high resistance rate observed for all *Enterococcus* sp. regardless of the isolation site, results from other study indicate that the majority of the vancomycin resistance enterococci were resistant to at least two of the tested antimicrobial agents besides vancomycin **(Iversen et al., 2002).**

From this study it can be demonstrated that bacteria that has been isolated from wastewater samples from Al-Shifa hospital and Laboratory building of IUG contain higher number of antibiotic resistant bacteria than bacteria that isolated from other sites in this study. Accordingly, previous studies have shown than waste effluent from hospitals contain higher levels of antibiotic-resistant enteric bacteria than waste effluent from other sources, hospital waste effluent could increase the numbers of resistant bacteria in the recipient sewer by both mechanisms of introduction and selection for resistant bacteria. (**Grabow & Prozesky 1973, Linton et al., 1974, Walterand & Vennes, 1985, Fontaine & Chopade, 1994)**

It is demonstrated that the resistance rate for some bacteria that isolated from the seawater sample is generally high particularly for *Pseudomonas* sp. Isolates, one study reported that river which is contaminated by treated

wastewater with many kinds of pollutants, is also contaminated with antibiotic resistant bacteria **(Iwane et al., 2003).**

## CONCLUSION AND RECOMMENDATION:

From the present investigation we can conclude that the release of wastewater from the hospital under study was associated with an increase in the prevalence of antibiotic resistance.

It is generally agreed that the selection and dissemination of resistant bacteria in nature should be avoided in order to ensure effective treatment against infectious disease in humans and maintain an ecological balance that favors the predominance of a susceptible bacterial flora in nature. According to the results of this study, factors other than the indiscriminate use of antibiotics in human medicine, animal husbandry, and agriculture may disrupt the microbial balance in favor of resistant bacteria .

## REFERENCES:

1. Al-Ahmad, A.; Daschner, F. D. and Kümmerer, K. (1999): Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of wastewater bacteria. Arch. Environ. Contam. Toxicol. 37, pp:158-163 (1999)
2. Astal, Z; El-Manama, A. and Sharif, F.A., (2002): Antibiotic resistance of bacteria associated with community-acquired urinary tract infections in the southern area of the Gaza Strip. Journal of Chemotherapy, Vol. 14, No. 3, pp: 259-264.
3. Bauer AM, Kirby WMM, Sherris JC and Turck M. (1966): Antibiotic susceptibility testing by a standard simple disk method. Am J Clin Pathol; 45, pp:493-6.
4. Blanch, A. R., J. L. Caplin, A. Iversen, I. Kühn, A. Manero, H. D. Taylor, and X. Vilanova. (2003): Comparison of Enterococcal populations related to urban and hospital wastewater in various climatic and geographic European regions. J. Appl. Microbiol. 94, pp:994-1002.
5. Baya, A. M. et al. (1986): Coincident plasmids and antimicrobial resistance in marine bacteria isolated from polluted and unpolluted atlantic ocean samples. Appl. Environ. Microbiol. 51, pp:1285-1292
6. Chitnis V, Chitnis D, Patil S, and Kant R., (2000): Hospital effluent: A source of multiple drug-resistant bacteria. Current Science, Vol. 79, pp:989-991.

7. Fontaine T., III and Hoadley, A. (1976): Transferable drug resistance associated with coliforms isolated from hospital and domestic sewage. Health Lab. Sci. 13, pp: 238-245 .
8. Fontaine, T and Chopade A. (1994): High levels of multiple metal resistance and its correlation to antibiotic resistance in environmental isolates of Acinatobacter. Biometals 7, pp:67-74.
9. Foster, T. J. (1983): Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol. Rev. 47, pp: 361-409.
10. Grabow, W and Prozesky, O. (1973): Drug resistance of coliform bacteria in hospital and city sewage. Antimicrob. Agents Chemother. 3, pp:175-180 .
11. Guardabassi, L., A. Petersen, J. E. Olsen, and A. Dalsgaard. (1998): Antibiotic resistance in Acinetobacter spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. Appl. Environ. Microbiol. 64, pp:3499-3502
12. Harwood, V. J., M. Brownell, W. Perusek, and J. E. Whitlock. (2001): Vancomycin-resistant *Enterococcus* spp. isolated from wastewater and chicken feces in the United States. Appl. Environ. Microbiol. 67, pp:4930-4933.
13. Iversen A, Franklin A and Kuhn I., (2002): High prevalence of vancomycin-resistant enterococci in Swedish sewage. Journal of Applied and Environmental Microbiology, 6, pp: 2838-2842.
14. Iwane T, Urase T and Yamamoto K. (2001): Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. Water Sci Technol. 2, pp: 91-99 .
15. Kruse, H.(1999): Indirect transfer of antibiotic resistance genes to man. Acta Vet. Scand.92 (suppl.), pp:59-65 .
16. Kümmerer. K and Henninger. A., (2003): Promoting resistance by the emission of antibiotics from hospitals and household into effluent. Clinical Microbiology and Infection, 12, pp: 1203.
17. Linton, K. B., Richmond, M. H., Bevan, R. and Gillespie, W. A. (1974): Antibiotic resistance and R factors in coliform bacilli isolated from hospital and domestic sewage. J. Med. Microbiol. 7, pp:91-103 .
18. National Committee for Clinical Laboratory Standards. (2001): Performance standards for antimicrobial susceptibility testing. Eleventh Informational Supplement. Document M100-S11. (2001) 21, No. 1. NCCLS, Wayne, Pennsylvania, USA.
19. Pathak, S. P., Gaur, A. and Bhattacherjee, J. W. (1993): Distribution and antibiotic resistance among aerobic heterotrophic bacteria from rivers in relation to pollution. J. Environ. Sci. Health A28, pp:73-87 .

20. Reinthaler, F.F., Posch, G. Feierl, G. Wust, D. Haas, G. Ruckenbaur, F. Mascher, and E. Marth. (2003): Antibiotic resistance of *E.coli* in sewage and sludge. Water Res. 37, pp:1685-1690.
21. Stuart. B., (2002): Factors impacting on the problem of antibiotic resistance. Journal of Antimicrobial Chemotherapy,1, pp:25-30.
22. Schwartz T, Kohnen W, Jansen B , and Obst U., (2003): Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol. Ecol .
23. ،43pp:325-335.
24. Vandepitte, A; El-Nageh, M.M.; Stelling, J.M.; Tikhomirov, E. and Estrela, A. 1996): WHO Regional Publications, Eastern Mediterranean Series. vol 15. Alexandria, Egypt: WHO Regional Office for the Eastern Mediterranean; 1996. Guidelines for Antimicrobial Resistance Surveillance.
25. Walter, M. V., and J. W. Vennes. (1985): Occurrence of multiple-antibiotic-resistant enteric bacteria in domestic sewage and oxidation lagoons. Appl. Environ. Microbiol. 50, pp:930-933.
26. Wegener, H., Aarestrup, F., Gerner-Smidt, P. and Bager, F. (1999): Transfer of resistant bacteria from animals to man. Acta Vet. Scand. 92 (suppl.), pp:51-58 .
27. World Health Organization (2002): Antibiotic resistance. WHO Media center WHO/Geneva. http://www.who.int/mediacentre/factsheets/fs194/en.