Identifications of multi-drug resistanturopathogens among infected patients from hospitals in Nablus-Palestine

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Abstract

Urinary tract infection UTI is the most common bacterial infection in the human population, and is considered to be a common cause of death all over the world. The increase in resistance in many bacterial strains prompted the researchers to do studies on it as well.

This research aims to investigate the multi-drug resistant uropathogens among patients taking their care in Rafidia and Al Watani Hospitals and Patients Friends Society (Mostawsaf Al-Rahma) in Nablus-Palestine using the skills and knowledge that we have acquired during study at ANNU to apply it in our graduating project from January to July 2021 in Nablus, Palestine.

100 urine samples from UTI infected patients have been randomly selected from the medical laboratories related to health care facilities mentioned above after the approval of IRP and the directorate of Health Education. The bacterial species have been identified then their multidrug resistant activity has been investigated against several numbers of antibiotics.

E.coli was the most frequent bacteria presented in the urine samples 39%, followed by *S.epidermidis*, *Enterococcus, K.pneumoniae, and Pseudomonas* respectively.

Frome the gram-positive group 4 samples were resistante to the entire antibiotics being used, and in general the samples showed the highest resistance for ampicillin, followed by amikacin, ciprofloxacin, Gentamicin, Imipenem.

From the gram-negative group 2 samples were resistante to the 5 antibiotics being used, and generally the samples showed the highest resistance for ciprofloxacin, followed by ceftazidime, gentamicin, imipenem, amikacin.

Introduction

Urinary tract infections are among the most common bacterial infections worldwide acquired in hospitals and the community affecting millions of people every year. With 30 times higher incidence in females due to their urinary tract anatomy [1–3], some statistics showed that 50% of females would have at least one UTI at some stage in their life [4].

UTI in healthy individuals that do not have any abnormalities are generally self-limiting condition but tend to recurrent [5].

The infecting microorganisms are identified by urine samples culture, the presence of 10^5 or more cfu/mL in urine, sample cultures are considered significant bacteriuria; however, if the samples are obtained by a sterile procedure (suprapubic aspirate or catheterization) from any patient with lower UTIs, colony counts lower than 10^5 cfu/ mL are significant [6].

UTIs can happen in different parts of our urinary tract. Each type has a different name, based on where it is [7]:

1. Cystitis (inflammation of the bladder).
2. Pyelonephritis (inflammation of the kidneys).
3. Urethritis (inflammation in the urethra).

Many studies showed that most cases of UTIs were due to the colonization of the urogenital tract with rectal and perennial normal flora. The most common organisms that cause UTI include (Escherichia *coli, Enterococcus, Klebsiella, Pseudomonas*, and other *Staphylococcus* species). *Candida* can also colonize the urogenital tract of some patients, especially in diabetic patients and patients with any immuonocompromise conditions [3, 4, 8].

Although the treatment of UTIs with antibiotics leads to a more rapid resolution of symptoms and is more likely to clear bacteriuria, it is also found to select for resistant uropathogens and commensal bacteria, this could harm the gut and vaginal microbiota [5, 6].

Nowadays, many bacterial strains have gained a resistance mechanism against many types of existing antibiotics.

The emerging of multidrug resistant (MDR) uropathogens has made the treatment of UTI cases very difficult, which may cause many complications for the patients if not treated [9].

Today, resistance to nearly all antimicrobial classes is dramatically growing, and extremely drug-resistant or even pan-drug resistant pathogens, are increasingly isolated around the world [10].

Since *E.coli* is the most common cause of UTI worldwide, many studies have been done on this bacteria to find the resistant isolates and their prevalence, and the level of resistance was found to be variable from one country to another, but with an increasing rate of resistance to antibiotics, careful monitoring of their use for UTI treatment is necessary [11–15].

A Palestinian study on *E.coli* showed a widespread of resistant strains due to the production of extended-spectrum β-lactamases and found that about 76 % of the isolates were noted to be multiply resistant [16, 17], Another Jordanian study found that 57.3% of *E.coli* isolates are MDR [13].

Several studies at the global level found plasmid-mediated antibiotic resistance among uropathogens [18, 19], and other studies showed a high percentage of multidrug- resistant uropathogens [20–22].

Multidrug-resistant infections are becoming a major source of in-hospital mortality and morbidity [4], A study in Palestine among ICU patients with sepsis found that the average mortality rate was 39.7% with UTIs being the second most common cause after respiratory infections [23].

We as medical laboratory students want to identify the bacterial species that were multidrug resistance among UTI-infected patients in Nablus between Januaryand July of 2021.

Method

- Sitting:

The study was conducted in both Rafedia and Al-Watany hospitals in addition to Patient's Friends Society (Mostawsaf Al-Rahmeh) in Nablus,Palestine,during the COVID-19 pandemic.

- Methodology:

The study was done on 100 purposive urine samples from UTI patients in Rafedia, Al-Watany hospitals, and the Patient's Friends Society (Mostawsaf Al-Rahmeh) in Nablus, Palestine.

To achieve that ethically, approval was sought from Institutional Review Board (IRB), and Directorate of Health Education, all the research protocols were performed under supervision of the laboratory supervisors in the hospitals and our Department Microbiology Lab at An-Najah National University [no. 17B1].

The urine samples were collected in a special sample container with ice for sample preservation during transporting from the hospitals to the ANNU lab, with an average of 8–10 samples per day taking our and community safety into consideration .

Samples were inoculated immediately after arriving at the university lab on blood and MacConky agar plates after mixing them at room temperature (25), the plates were placed in the incubator for 24 hours at 35–37°C.

On the next day, the bacteria from blood or MacConky plates were distinguished into gram-positive (the once that grown just on the blood agar) and negative (the once that grown on both blood and MacConky plates), and cultured on Uri select media for identification, after one day in the incubator the grown bacteria have been identified according to its characteristics and color referring to instructions and pictures from the manufacturer of the media, the ones that were questionable and not clearly identified from gram-positive; a catalase and coagulase tests were conducted to correctly identify them. while the ones from gram-negative group; an Analytical Profile Index (API) method were used to identify them. Confused bacterial community have been confirmed under the microscope (e.g. one of the samples was confused wheather it was *S.agalactiae* or *Candida albicans*, but under the microscope a coccus bacteria in strips was visualized so it was *S.agalactiae*, another one was suspected to be *E.coli* or *K.pneumoniae;* under the microscope a motile bacteria was visualized so its *E.coli*).

Before performing the antibiotic sensitivity test, a subculture for the samples on nutrient agar has been done to get newly, fresh growing bacteria to perform Disk Diffusion Method (DDM).

A bacterial suspension from the bacteria on nutrient agar with absorbance between 0.08–0.12 was done, from this suspension, using aseptic technique; broth culture of a specific organism was collected with a

sterile swab, the excess liquid was removed from the swab by gently pressing or rotating it against the walls of the tube.

A lawn culture was obtained by streaking the swab on Mueller-Hinton agar, and in order to have a uniform culture; the plate was streaked with the swab in one direction, then it was rotated 120° and was streaked again, was rotated another 120° and was streaked one last time.

After that, the antibiotics were placed on the plates, 5 antibiotics were used for gram-positive group (Ampicillin (AMP 10µg), Amikacin (AK 30µg), Imipenem (IPM 10µg), Gentamicin (CN 10µg), ciprofloxacin (CIP 5µg)) and another 5 for gram negative group (Imipenem (IPM 10µg), Gentamicin (CN 10µg), Amikacin(AK 30µg), Ceftazidime(CAZ 10µg), Ciprofloxacin(CIP 5µg)).

After one day the results were obtained in tables by measuring the diameter of the zone of inhibition around each disc of the antibiotics, and referring to disc diffusion supplemental tables, results were classified as resistance(R), intermediate(I), and susceptible(S) [24].

All the procedures were performed under aseptic conditions, and all COVID-19 safety rules were followed in all places, which involve masks, lab coats, and gloves.

The samples and plates were discarded according to the international safety role in autoclavable bags, see Fig. 1.

Results

From the 100 urine samples, 64 samples were identified as gram-negative bacteria (the bacteria grown on both blood and maCconky agars) and 36 samples were identified as gram-positive (the growth was only on blood agar for these samples), the main isolates were *E.coli*, followed by S.epidermidis, Enterococcus, K.pneumoniae, Pseudomonas aeruginosa, Proteus, Enterobacter, S.aureus, Serratia odorifera, and S.agalactiae, the percent of each one shown in Fig. 2 below.

The sensitivity test results were arranged in tables 1 and 2).

4 species of the gram-positive samples (2 *S.epidermidis* and 2 of *Enterococcus)* and 2 of the gram-negative (one was *Pseudomonas aeruginosa* and the other was *E.coli*) were found to be resistant for the entire 5 antibiotics been used for each, see Fig. 3 below.

Even though some of the samples were found to be susceptible to the antibiotics being used, other samples showed resistance to more than 2 antibiotics.

From the gram-positive group, 9 samples have resistance for 1 of the antibiotics (samples number 10, 21, 28, 36, 42, 47, 86, 97, 98), 11 of the samples showed resistance for 2 of the antibiotics(no. 5, 6, 19, 24, 43, 51, 56, 65, 84, 94, 95), 1 of them had resistance for 3 antibiotics (no. 29), and 2 have for 4 antibiotics (no. 3, 7).

From the gram-negative group, 16 samples have resistance for 1 of the antibiotics (samples number 8, 9, 11, 14, 25, 35, 46, 57, 64, 67, 69, 71, 82, 88, 91, 100), 13 samples showed for 2 antibiotics (no.13, 15, 20, 23, 48, 59, 60, 70, 80, 83, 92, 93, 99), 4 samples showed resistance for 3 (no. 38, 53, 58, 68), and 2 of them showed resistance for 4 antibiotics (no. 61, 63).

Discussion

The results of this study showed that 40% of identified bacteria have resistance to some or all the antibiotics being used which has been agreed with several studies [25, 26].

What is interesting is that the gram-positive bacteria samples had many bacteria that were resistant to all the antibiotics more than the gram-negative group, even though the gram-negative is more common to cause UTI, because the gram-negative bacteria is the predominance bacteria in the gut flora and other structures [6], as you can see that the number of gram-negative samples (64) is more than the gram-positive (36).

As it is known globally, *E.coli* is the most common bacteria that cause UTI. The results showed that *E.coli* is the most frequent bacteria found in the urine samples, and from the gram-positive group *S.epidermidis* was the most common one [2, 6, 27].

Susceptibility testing is usually performed to evaluate which antimicrobials will stop the growth of the bacteria or fungi that are causing infection and kill them. The findings of this test will assist a healthcare provider in determining which drugs are most likely to be beneficial in treating an infection.

In our study In the gram-positive group, the bacteria showed the highest resistance for AMP (16 samples), followed by AK (15 samples), CIP (14 samples), CN (12 samples), and the lowest resistant was for IPM (5 samples).

In the gram-negative group, the bacteria showed the highest resistance for CIP (23 samples), coming next was CAZ (18 samples), CN (17 samples), IPM (11 samples), and the lowest resistance for AK (3 samples).

The results confirm wrong health practices and lack of health awareness among citizens, one of them the misuse of antibiotics without consulting the doctor and easy purchasing of antibiotics from pharmacies without doctor prescriptions. Also, some doctors do not ask for an antibiotic susceptibility test to see which antibiotic is the best to use before prescribing medication for the patient. Therefore, the awareness of rationalization of use of medicines and antibiotics should be spread over [6].

In the last few years, the resistance of gram-negative bacteria has been increasing due to the appearance of new strains that produce extended-spectrum β-lactamases (ESBLs) [28].

Recommendations:

More efforts should be done to contain multidrug resistance uropathogens as well as strains identification in addition to awareness strategies of health policies for health care practionares and Palestinian community health regarding multidrug resistance uropathogens and related practices like the risks of dispensing medication without a prescription.

Limitations:

Limitation concerns Covid-19 and the Palestinian Ministry of Health Workers strike that delayed the approval paper to start work on the project. In addition to difficulty in obtaining several samples in the first two months due to the doctors' syndicate strike.

We had another difficulty in dealing with the staff at some hospitals as some of them were not cooperative.

Declarations

Conflicts of Interest

The authors declared that they have no competing of interests in this manuscript's publication.

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Authors' Contributions

Dr Wafaa Menawi has designed the study and supervised the action plan and manuscript writing and finalization. Lama Zaid,Tamador Athamny, Lama Khodair, Rahaf Zain Al Deen, Mais Shehadeh and Haya Mansour have collected the samples and conducting all the manual tests to answer the question of the study as well as writing the manuscripts. All of the authors have read and approved the final version of the paper.

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Abbreviations

UTI: Urinary Tract Infection

ANNU: An-Najah National University

IRP: Institutional Review Board

MDR: Multi-Drug Resistance

ICU: Intensive Care Unit

API: Analytical Profile Index

DDM: Disk Diffusion Method

AMP: Ampicillin

AK: Amikacin

IPM: Imipenem

CN: Gentamicin

CIP: Ciprofloxacin

CAZ: Ceftazidime

R: Resistance

I: Intermediate

S: Susceptible

ESBLS: Extended-Spectrum B-Lactamases

E.Coli: Escherichia Coli

S.Epidermidis:Staphylococcus Epidermidis

S.Aureus: Staphylococcus Aureus

S.Agalactiae:Streptococcus Agalactiae

K.Pneumoniae:Klebsiella Pneumonia

S.Odorifera: Serratia Odorifera

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