ISSN: 03875547
Volume 44, Issue 06, December, 2021

Evaluate Multi-Drug Resistance Pattern of Microbial Flora from Hospital Premises of Baghdad Hospitals

Ahmed Najm Abed[1*], Omar Ahmed[2], Modni Abdulmajeed[1], Yasmine Mustafa Khazaal[1]

Department of Nursing, Al-Esraa University college, Baghdad, Iraq[1]
Department of Bioinformatics, Biomedical informatics College, University of information technology and communication, Baghdad, Iraq[2]

Corresponding Author: 1*

Keywords:
Multi-Drug Resistance, Microbial Flora, Klebsiella oxytoca

ABSTRACT

The current study was carried out in order to gain an understanding of the diverse microorganisms linked with trash dump soil and their antimicrobial resistance profiles in four hospitals in Baghdad. All the isolates' morphological, biochemical, and physiological features were analyzed. A systematic morphological, and biochemical identification was carried out once isolated colonies were isolated from each sample. From the results obtained it was observed that *Klebsiella oxytoca. Staphylococcus aureus, E.coli, Providentia* were predominant organisms. The standard disc diffusion method was used to perform antibiotic susceptibility testing for isolates. We studied the three major organisms found in the soil namely *staphylococcus, e.coli* and *klebsiella*. Fortunately most of them were sensitive to the common drugs used in hospitals except for *klebsiella*, which showed weak resistance to the drug piperacillin. Ciprofloxacin was the most potent agent on the isolated bacteria, followed by Augmentin and Gentamicin in hospital solid waste, respectively. Most of the isolates were sensitive to common drugs. DNA was extracted from every organism isolated in this study and amplified via PCR followed by purification and Genome sequencing. The sequences were then analyzed to identify the bacterial isolate.

1. INTRODUCTION

A little scoop of soil includes a big library of genes that code for proteins that help bacteria flourish in their natural habitat. New studies have shown that certain genes that drive human bacteria to resist antibiotic treatment are identical. The results shown that the original cause of certain antibiotic-resistant genes in hospitals is caused by healthy soil bacteria. In soil-based bacteria throughout millions of years, natural antibiotics often created by opposing organisms are introduced and resistance mechanisms through coevolution also are established. Penicillin is the most popular illustration. Because bacteria are known to swap genes when they come into contact, researchers theorized that specific soil resistance genes might make their way to microbial pathogens like E.coli or Staphylococcus in people and animals. Nonetheless, screening the soil sample for distinct gene variations is a challenge due to the large number of genes that have been documented. The rest of the history has been influenced by the bacterial tolerance genes found in soil [1]. Antibiotics have played an important role in ensuring public safety since the 1940s, and they have

saved millions of lives. Unfortunately, the overuse of antibiotics threatens their effectiveness. Since current food animal factories use low amounts of antibiotics, drug-resistant bacteria have emerged on farms, infiltrating the general population via human and animal carriers and consuming food customers [2]. Antibiotics are passed through poultry into poultry and even from poultry toward humans. Plasmids are the indicator that shows the spread of antibiotic resistance passing through poultry into poultry and even from poultry toward humans [2]. Because of the risk of antibiotic resistance to public health, small doses of medically necessary antibiotics are being avoided with caution an attempt to delay the development of antibiotic-resistant bacteria [3].

Antibiotic resistance develops because of the overuse of antibiotics for viral symptoms such as influenza or the common cold, which an antibiotics do not treat. Antibiotic resistance can develop even when drugs are used to treat bacterial illnesses, according to studies. Failure to complete the prescribed medicines regimen for bacterial illness is another crucial factor in the developing antibiotic resistance [4].

2. Methodology

2.1 Clinical isolation and identification

Soil and water samples were collected from Al Yarmuk General Teaching hospital, Al Qadisiya General Hospital, Dejla Private Hospital, And Abdul Majeed Private Hospital premises in November 2020. In the laboratory for microbiology, the obtained samples have been aseptically processed. For this project, a variety of media types were used. Each medium varies in its components to promote the production of a particular pathogen. Merck Millipore, Germany, provided the media and reagents used in the study, including Nutrient Broth, Nutrient agar, Blood agar, Eosin-Methylene Blue agar, Thiosulfate-Citrate-BileSalt-Sucrose agar, Salmonella-Shigella agar, Mannitol Salt agar, Methyl red, MacConkey agar, as well as Voges Pro. Incubate the prepared dishes for 48 + 2 hours, at 35oC. Bacterial a count was taken three times on solid Nutrient Agar plates after serial dilution from 1 in 10 concentration levels on all samples using the Lazy Susan plating technique. The plates have been sealed and incubate at 37 0 C. The microorganisms that created colonies were calculated after 24 and expressed in CFU/mg. Smears were created from single colonies on plates in order to classify each microorganism and stained by grams to determine if were grams negative or grams positive. Motility testing was performed for bacterial isolates. Bacterial pathogens have been isolated and identified based on their pattern of growth and colonization characteristics in selective and differential media. IMViC, oxidase, catalase, urease, and motility tests were also conducted to verify results in accordance with standard laboratory procedures.

2.2 Antibiotic susceptibility tests

The culture media have been prepared with industrial powder reconstruction in distilled water and sterilized by autoclave at 121 ^{o}C for 15 minutes [5]. On Hicrome agar media, the isolated microbial species were cultivated. The isolates were analyzed according to the national Clinical laboratory Standard Guidelines by disc-diffusion method for drug susceptibility [6]. This was then distributed antimicrobial disks and pressed down onto agar carefully by sterile forceps. The antibiotics gentamicin (10ug), ampicillin (10ug), tetracycline (30ug), penicillin (10ug), ciprofloxacin (5ug), and ceftazidine (30ug) from Sigma-Aldrich were used in this study. The plate was then incubated for 24 hours at 370°C, and after that, the inhibition zone was determined. Antimicrobial criteria evaluated and interpreted according to CLSI 2016 [7].

2.3 DNA extraction and PCR sequencing

The Isolated bacterial were plated on Hekton Enteric Agar and cultivated for 24 hours at 37oC in the incubator, and these bacterial cultures were used for DNA extraction. APrep man kits have been used for

the isolation of bacterial DNA, this method was developed via anonymous 2000 [8]. The mix of PCR contains 1mL Bacterial DNA in sterile 500 mL micro-centrifugal tube 1 mL each rP2 primer5'ACGTTAAAGGCCTTAGGGCCCAAATT-3 as well as fD1 primer 5'-CGAGGGCCCTTAAATTGGCCAA TAAG-3. The Master PCR (Shimadzu) 12.5mL HotStar mixed and 9.5 mL distilled water was added to the reaction mix in the PCR device. The PCR component was moved in a 2.5% agarose gel of electrophoresis, and figure (1) shows the results for purification and DNA sequencing, samples displaying an appropriate size band had been preserved. The QIAquick PCR purification Kit (Qiagen Valencia, CA) was used to purify PCR components and elute them with water. DNA sequences have been examined for identification, finally tabled in the table (4), by submission to the website of the ribosomal database project [9].

3. Result

Approximately five grams/5 ml of every sample was poured into TSB broth and incubated 24 hours at 37 0c. The samples were diluted 10 times the next day, and the fifth and sixth dilution samples were plated on LB agar to obtain pure cultures. Each sample's isolated colonies were chosen and streaked onto a sterile nutrient agar plate, the master plate, and kept at 4 0c. Then every time, a master plate was sub-cultured in order to perform different tests. Gram staining and motility experiment have been used to identify each isolated colony on the master plate table (1). IMVIC tests were done according to the standard laboratory procedures, and results are recorded in table (2).

Table 1. morphology properties and gram staining of isolated bacteria

Characteristics	*Klebsiella oxytoca*	*E.coli*	*Staphylococcus aureus*
Colony shape	Round and large	Round and small	Round
Shape	Rods	Rods	Cocci
Gram stain	Gram negative	Gram negative	Gram positive
Pigmentation	Rose-pink	Grayish white	Golden-gray
Texture	Mucoid	Mucoid	Smooth
Margin	Undulate	Entire	Entire
Opacity	Opaque	Opaque	Opaque
Elevation	Raised	Slightly raised	Convex

Table 2. Biochemical test of isolated bacteria

Biochemical tests						
Indole	**VP**	**MR**	**Citrate**	**Catalase**	**TSI**	**Organism**
(+)	(-)	(+)	(-)	(+)	(-)	*E.coli*
(-)	(+)	(+)	(+)	(+)	(-)	*S.aureus*
(+)	(+)	(-)	(+)	(+)	(+)	*klebsiella*

3.1 Antibiotic susceptibility test

Different antibiotic resistance profiles were shown in table (3). In general, E.coli showed sensitivity to all antibiotics, Klebsiella oxytoca showed full resist to Cefuroxime 30 mg, and Ampicillin 10mg, and weak resist to Piperacillin 100µg, Ciprofloxacin 5µg and Gentamycin 10µg, Staphylococcus aureus showed weak resist to Cefuroxime 30µg Teicoplanin 30 µg, and Cefotaxime 30 µg.

Table 3. Different antibiotic resistance profile

Antibiotics	*Klebsiella oxytoca*	*E.coli*	*Staphylococcus aureus*
Cefuroxime 30 µg	Resistance	Sensitive	Weak resist
Piperacillin 100 µg	Weak resist	Sensitive	Sensitive
Imipenem 10 µg	Sensitive	Sensitive	Sensitive
Meropenem 10 µg	Sensitive	Sensitive	Sensitive
Ciprofloxacin 5 µg	Weak resist	Sensitive	Sensitive
Cefoxitin 30µg	Sensitive	Sensitive	Sensitive
Vancomycin 30 µg	Sensitive	Sensitive	Sensitive
Erythromycin 15 µg	Sensitive	Sensitive	Sensitive
Teicoplanin 30 µg	Sensitive	Sensitive	Weak resist
Tetracycline 30 µg	Sensitive	Sensitive	Sensitive
Cefotaxime 30 µg	Sensitive	Sensitive	Weak resist
Gentamycin 10 µg	Weak resist	Sensitive	Sensitive
Ampicillin 10 µg	Resistance	Sensitive	Sensitive

3.2 PCR result

PCR gel analysis samples showed isolates bands according to reference sample with the predicted size among 100 and 1200bp figure (1). Sequencing soil and water samples produced consistent results, and was matched database organisms, the result was tabulated in Table (4).

ISSN: 03875547
Volume 44, Issue 06, December, 2021

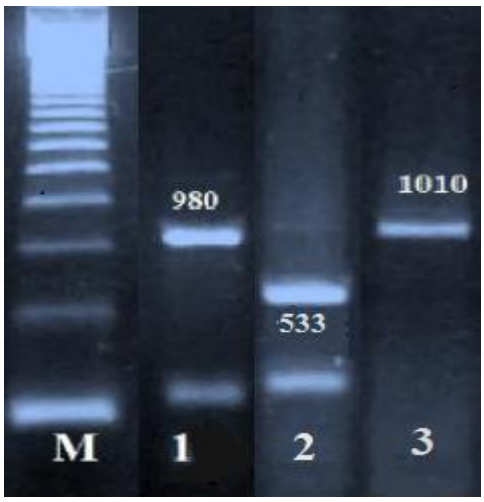

Figure 1. PCR gel analysis sample showed bands to the sample

Table 4. Sequences sample data base

Bacterial Isolate	5'-3' sequence
E.coli	CCACACATCAATTACCTAACACGGTCCGCAATAGCGATATCCG TTTCGCGCG CAAATTCCCTAAGCCCTAAGGGCCAAT
S.aureus	CCTTTGGCCCGTGCACCCTTAAGGGATTCCCAAGTTTAACATA GGCCTTTAACCCGGACCAATATTTGGCAAAGGCCCGG
Klebsiella oxytoca	GCTAACATGGCCAATTCAACCCATTAACTTGGGGCCCTTAAAC CCTTCCCAAATTTCCGGAAAGGCCCGGAAATTTAGGGT

4. Discussion and Conclusion

The medicines are so commonly used and have been used for such a long time that the infectious organisms have adapted to the antibiotics, making the medication less effective. The same thing has happened with fungi, viruses and parasites. Several microorganisms show tolerance to a single antimicrobial drug, while some others develop resistance to different antimicrobial drugs. The above pathogens are also known as multi-drug resistant microorganisms or MDR pathogens. Pathogens have become so tolerant in some circumstances that no antibiotics are effective against them. Antibiotics are being utilized in large amounts, and as antibiotic resistance rises, there is an urgent need to create new and creative antimicrobial drugs. Plants having long been studied as potential sources of novel agents against resistant pathogenic strain [10]. There is a long history of employing dietary plants in the treatment of infectious diseases and the successful struggle against MDRs because they contain various bioactive chemicals that may be of therapeutic interest and because of their low toxicity. Tetracycline resistance is an appealing model for investigating antibiotic resistance. Tetracycline's antibacterial activity is accomplished by attaching to the bacterial ribosome and blocking protein synthesis. This has been employed not just in veterinary and human medicine, but as a growth booster also in animal husbandry [11].

5. References

[1] Abulreesh, H. H. (2011). Multidrug-resistant staphylococci in the environment. In International Conference on Biotechnology and Environment Management (Vol. 18, pp. 1-6).

[2] Levy, S. B., Fitzgerald, G. B., & Macone, A. B. (1976). Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. Nature, 260(5546), 40-42.

http://www.cdc.gov/drugresistance/solutions-initiative.

[3] Milene Fernandes etal,, Andreia Leite, Maria Basto, Miguel Araújo Nobr, Nuno Vieira,Rui Fernandes, Paulo Nogueira, Paulo Jorge (2014) International Journal of Clinical Pharmacy Volume 36, Issue 1, pp 86-91

[4] Onyango, D., Machioni, F., Kakai, R., & Waindi, E. N. (2008). Multidrug resistance of Salmonella enterica serovars Typhi and Typhimurium isolated from clinical samples at two rural hospitals in Western Kenya. The Journal of Infection in Developing Countries, 2(02), 106-111.

[5] Cormican, M., Whyte, T., & Hanahoe, B. (2001). Antimicrobial susceptibility testing in Ireland: An introduction to the methods of the National Committee for Clinical Laboratory Standards (NCCLS). Department of Medical Microbiology, University of Colleg Hospital Galway and Department of Bacteriology. National University of Ireland Galway.

[6] Wayne, P. A. (2011). Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.

[7] Anonymous, Prepman Ultra Sample preparation reagent quick card, Applied Biosynthesis, Foster City, CA, 2000.

[8] http://rdp.cme.msu.edu/.

[9] Grabow, W. O. K., & Prozesky, O. W. (1973). Drug resistance of coliform bacteria in hospital and city sewage. Antimicrobial agents and chemotherapy, 3(2), 175-180.

[10] Nahid Karami*, Forough Nowrouzian, Ingegerd Adlerberth and Agnes E. Wold Antimicrob. Agents Chemother.January 2006 vol. 50 no. 1 156-161.

[11] van den Bogaard, A. E., & Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics: links between animals and humans. International journal of antimicrobial agents, 14(4), 327-335.