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Article

A study on the effectiveness of antibiotics against bacteria isolated from the Al-Khassa river in Kirkuk city, Iraq

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ABSTRACT
Sewage uses wastewater consisting of human excreta, wash waters, and industrial and agricultural wastes (e.g., wastes from livestock, i.e., chicken, cattle, and horse) that enter the sewage system. The study aims to study bacteria isolated from sewage water to know their source and different antibiotics used and compare it with other bacteria present in human excretion. This study was carried out in March - 2019. 30) Water samples were taken from the Al-Khassa River in Kirkuk, starting from the Al-Tabaqchary bridge to the Domiez bridge; all the samples were worked in the general health laboratory of Kirkuk. All the water samples were collected in sterile containers and were processed within one hour after arriving at the laboratory. First, it was cultured on blood and MacConkey agar for each sample; the petri dishes were put in an aerobic incubator at 37 ^{C0} for 24 hours. It was isolated on nutrient agar for purification, incubating at 37 ^{C0} for 24 hours. Bacterial colonies were identified based on diagnosis using morphology, cultured, and biochemical characteristics. The antimicrobial screening was performed using Muller-Hinton agar disk diffusion assay for signing drug sensitivity; the study showed that E. coli represented the most isolated bacteria from Al-Khassa River (14 isolates), Serratia marcescens (10 isolates), Pseudomonas aeruginosa (5 isolates) and Proteus spp. (only 2 isolates), The study showed that gentamicin was the most active antibiotic against the Gram-negative bacilli isolates tested, as only 2.6% of these isolates were resistant to this antibiotic at concentrations up to 10µg while susceptible to clindamycin.

Keywords: Al-Khassa; Kirkuk; Sewege, river; Contamination

INTRODUCTION

Sewage uses human excreta, wash waters, and industrial and agricultural wastes (e.g., wastes from livestock, i.e., chicken, cattle, horse, etc.) that enter the sewage system. Sewage generally contains about 95.5% water and 0.1 to 0.5% organic and inorganic materials. The solid continues to exist in the water in a suspended state [1,2]. Colloidal forms of celluloses, lignocelluloses, proteins, and fats can be found in water. The remaining inorganic materials dissociate into their ionic states and are found to be dissolved [3]. In northern Iraq, Kirkuk is traversed by the Khassa River, a river with a Winterbourne source that runs through the city. It is

scorched during the summer months, but during the winter months, it transforms into a raging river that occasionally overflows its banks, as in the 1950s. Residents of the city attach a certain amount of significance to the river. It is a river that flows into the Tigris and contributes to its flow [1].

Numerous pathogens, such as bacteria, fungi, protozoa, algal worms and viruses, can be found in water. Waste water must not be disposed of in a manner that is detrimental to human health and the environment. It is important to remember that our planet can heal itself and that we should always aim to stay within that range [4,5,6]. The safest course of action to avoid this limit, which scientists are not always clear on, is what we must always follow: Organic and suspended solids are removed from wastewater to reduce pollution. Due to changes in requirements and technology, new methods for removing dissolved matter and toxic substances have been developed [7,8]. The advancement of science and morality has led to decreased emissions through pollution prevention and recycling to achieve the noble goal of zero pollution discharge [10,11]. Antibiotic-resistant strains of bacteria found in sewage water will be compared to those found in human excrement, as will the source of the bacteria.

MATERIALS AND METHODS

This study was carried out in March - 2019. 30) Water samples were taken from the Al-Khassa River in Kirkuk, starting from the Al-Tabaqchaly bridge to the Domiez bridge; all the samples were worked in the general health laboratory of Kirkuk. All the water samples were collected in sterile containers and were processed within one hour after arriving at the laboratory. First, it was cultured on blood and MacConkey agar for each sample; the petri dishes were put in an aerobic incubator at 37 ^{C0} for 24 hours. It was isolated on nutrient agar for purification, incubating at 37 ^{C0} for 24 hours. Using morphology, cultured characteristics, and biochemical characteristics such as (catalase, oxidase, coagulase, gelatinase test, triple sugar iron, indol test, methyl-red-Vogas proskauer, citrate test), bacterial colonies were diagnosed and identified based on the color chart, hemolysis, gas production, pigment formation, and microscopically with Gram's staining, depending on their shape and color by using oil immersion (At least five to ten high-power fields were scanned to diagnose bacteria, and the results were reported using forms. The antimicrobial screening was carried out with a Muller-Hinton agar disk diffusion assay to determine the drug sensitivity level. The antibiotic discs were placed on the bacterial culture with a sterile wooden stick. The following antibiotics, Cefotaxime, Ceftazidime, Levofloxacin, Azithromycin, Amikacin, Gentamycin, Tetracycline, Norfloxacin, and Flagyl, were put to the test. The results of sensitivity were observed following a period of 24 hours of incubation at 37Co. Antimicrobial activities were measured using a caliper to determine the inhibition zone diameter around the disc.

RESULTS

The study showed that E. coli represented the most isolated bacteria from Al-Khassa River (14 isolates), Serratia marcescens (10 isolates), Pseudomonas aeruginosa (5 isolates) and Proteus spp. (only 2 isolates), Table 1.

Bacterial species	Total No. of isolates
Escherichia coli	14
Pseudomonas aeruginosa	5
Serratia marcescens	10
Proteus spp.	2

Table 1. Gram-negative bacterial species isolated from sewage samples from the Alkhassa river

After that, the susceptibility of the four identified Gram-negative bacilli to each of the seven antibiotics that were being evaluated was determined. The data presented in Table 2 show that gentamicin was the most active antibiotic against the tested Gram-negative bacilli isolates.

Bacterial species	No. of isolates	Gent 10 µg	Cef 30 µg	Amp 2 µg	Cefa 10 µg	Cipro 30 µg	TMP 1.5 µg	Tet 25 µg
Escherichia coli	13	11	8	7	1	1	3	12
Proteus vulgaris	10	10	5	–	–	–	–	10
Pseudomonas aeruginosa	5	4	3	3	2	4	1	1
Serratia marcescens	2	2	1	–	–	2	1	2

Table 2. Resistance of Gram-negative bacterial isolates to different antibiotics.

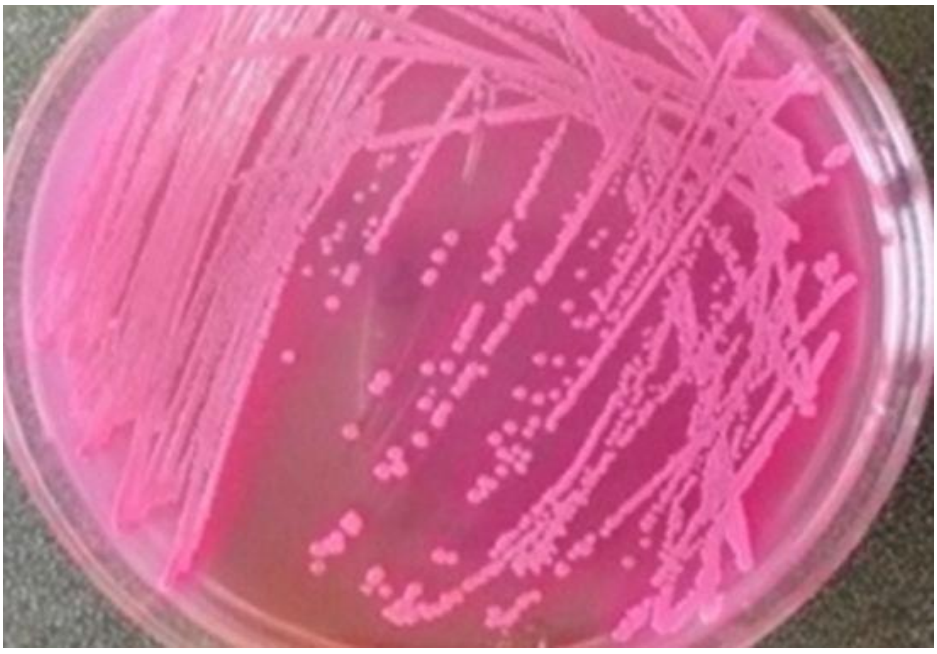

Figure 1. E coli on MacConkey isolated from Al-Khassa river

Figure 2. E coli on CLED agar isolated from Al-Khassa river

Figure 3. Growth of Serratia marcescens

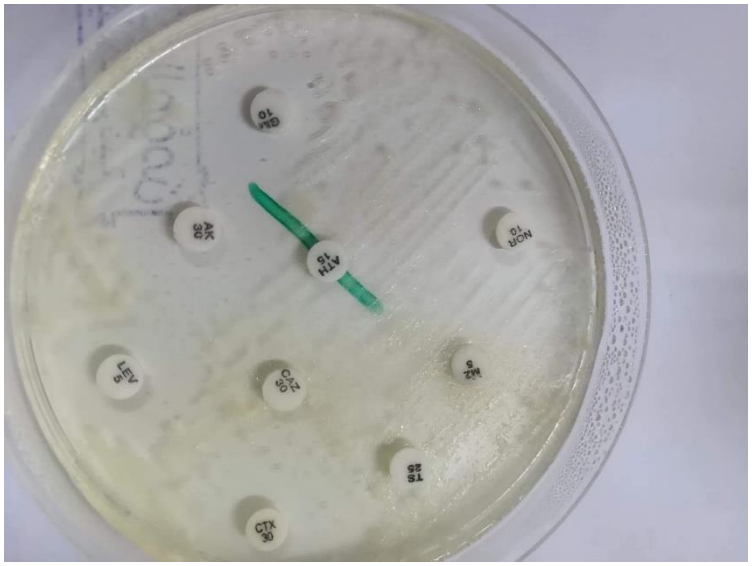

Figure 4. Example of antibiotic sensitivity test done on bacterial isolates

DISCUSSION

In our finding, except for Gram-negative bacilli, Gram-negative cocci were not found in the current investigation. In nature, Gram-negative cocci are extremely rare because of their demanding nutritional and environmental needs [12,13]. Four distinct genera of Gram-negative bacilli were identified, each with a wide range of genetic variation. Some genera include pathogens like Escherichia coli and Pseudomonas aeruginosa, while others do not (e.g., Serratia.). Because of the low persistence of the organism in unfavorable conditions, these findings are likely to be accepted for sewage samples subjected to various environmental conditions [14,15] and other studies [16,18]. According to research, hospital patients with compromised immune systems may be infected by organisms commonly found in sewage. They pose an increased risk to human health because of their ability to resist the effects of antibiotics, which are typically prescribed to treat the infections they cause [19,21]. As a result, it is even more critical to keep an eye out for pathogenic bacteria in sewage and to make the necessary efforts to eradicate them. The seven antibiotics were evaluated to see if any gram-negative bacilli were susceptible. In this experiment, saturated discs were used, and the concentrations tested were those commonly found in hospitals. Gentamicin was the most effective antibiotic against the Gram-negative bacilli isolates tested, according to Table 2 and the graph. Many medications are excreted by patients without being metabolized and end up in the sewer system [22] after they have been administered. At concentrations of 20 to 80 micrograms per liter of effluent, ampicillin was found in a large German hospital's effluent. The antibiotic concentrations in hospital effluents have been calculated and measured on the same order of magnitude as the minimum inhibitory concentrations for susceptible pathogenic bacteria [24]. Due to the presence of antibiotics and disinfectants from the household, veterinary, and, to a lesser extent, livestock sources in municipal wastewater, hospital effluents will only have a modest effect on reducing antibiotic concentrations. Antibiotics from hospitals are found in municipal wastewater, which is why this is the case. Microgram per liter concentrations of antibiotics have been found in municipal sewage effluents, surface water, and groundwater [25,28]. In 2003, [29] conducted a study to evaluate E. coli resistance patterns in wastewater treatment plants in southern Austria. [Reference required] According to [Citation required], [Reference required] [Citation required] There is a citation needed for this. [Cit There were 767 E. coli isolates tested for antibiotic resistance, and the results showed they were all resistant to 24 different antibiotics. E. coli strains with the highest resistance rates were discovered in a sewage treatment plant that treats municipal sewage and sewage from a hospital. Sewage from the city is also processed at this facility. Tests on antimicrobial agents revealed that ampicillin, piperacillin (up to 18%) and cephalothin (up to 12%) had the highest rates of resistance among the penicillin-group antimicrobial agents (up to 35 percent). In another study, ampicillin, chloramphenicol, and tetracycline showed the highest resistance levels among the isolates collected from hospitals and municipal wastewater. Antibiotic-resistant strains were frequently discovered in hospital and city wastewater (85 percent and 65 percent respectively). One-fifth of the strains from the two sources were resistant to six different antibiotics in multiple ways. Wastewater without treatment plays a significant role in the spread of resistant coliforms, which has serious implications for human health [16]. Salmonella strains isolated from sewage water, chicken, and food handlers in Oman were tested for antibiotic resistance. All 1242 food handlers isolates were resistant to one or more antibiotics. Antibiotics ampicillin, co-trimoxazole, and chloramphenicol

were all resistant to 41% of these organisms, while the remaining 42% were susceptible. Other researchers' findings [23, 29, 30] and ours back up the idea that antibiotics like ampicillin, whose effectiveness is still up in the air, should be restricted. Several studies have found ampicillin to be one of the antibiotics against which bacteria have developed the most resistance, thus the reason for this finding. The problem affects all regions of the world, not just those with less developed economies when the research from developing nations is compared to that from more developed nations. In particular, Gram-negative rods resistant to antimicrobial drugs containing beta-lactam have shown increasing resistance to antimicrobial agents. Cephamycins and broad-spectrum cephalosporins quickly developed resistance in Gram-negative bacteria. Plasmodium-encoded extended-spectrum lactamases (ESBLs), carbapenemases, cephamycinases, and cephamycinases were some of the mechanisms that led to the acquisition of this resistance. An MDR bacterial population in hospital effluents ranged from 0.58 to 40%. However, in 11 sewage samples from residential areas, the bacterial population was less than 0.00002 to 0.025 percent, according to a study looking into the spread of MDR bacteria from hospital effluents to the municipal sewage system. Hospital effluents may be hazardous to public health because of the addition of MDR bacteria to a city's sewage pool. Hospital effluent samples contained an alarmingly high number of MDR bacteria. Most antibiotics currently used to treat human infections were found to have an MDR pattern in bacterial isolates taken from hospital effluent samples. The most frightening prospect is that such resistance could be passed on to bacterial pathogens that cause infections in the community [32].

CONCLUSION

The findings of this current study make it abundantly clear that there is a problem with multidrug resistance among the pathogenic bacteria isolated from sewage water. The only way to get a handle on this problem, which is expanding at an alarming rate, is to have a rational approach to antibiotic policy. This is an introduction to another forthcoming piece of work that will investigate the use of bacteriophages in sewage treatment plants to control MDR pathogenic bacteria.

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