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THE EFFECT OF PH ON THE RESISTANCE OF PROTEUS MIRABILIS BACTERIA TO ANTIBIOTICS

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Abstract: Twenty-six bacterial isolates of Proteobacteria were isolated from patients with urinary tract infections from several hospitals in Babylon province. Urine samples were collected from patients with urinary tract infections, then these samples were grown on different culture media and identified by several biochemical tests. Also, their drug sensitivity was studied using nine types of antibiotics. The drug sensitivity of these samples was studied at acidic, neutral and basic pH using disk diffusion method. The study showed that ciprofloxacin and azithromycin had high activity against bacteria at both acidic and neutral pH, while amikacin, meropenem, imipenem and ciprofloxacin had high activity at basic pH against these bacteria

Keywords: -

Introduction

Proteobacteria

Urinary tract infection is a widespread and difficult problem in the world, affecting millions of people annually. Women are more likely than men to be affected by this disease. It comes in second place after respiratory tract infections, as both males and females are exposed to this disease, especially since it is one of the main causes of death in infants (1) P. mirabilis bacteria are opportunistic microorganisms belonging to the intestinal family, as they cause health problems for humans and animals alike (2), as they cause infections of the digestive system and the reproductive system. The medical importance of this bacteria comes after Escherichia coli bacteria and Klebsiella pneumonia bacteria, as they cause health problems and pathological injuries to the reproductive system, the digestive system, and other systems (3. (

Proteus bacteria cause urinary tract infections, especially the type P. mirabilis, and despite that, it comes after E. coli bacteria in causing urinary tract infections ((4 This bacteria causes urinary tract infections for patients lying in hospitals and users of urinary catheters for long periods, as well as people who suffer from functional problems and Abnormal urinary tract (5) The ability of this bacterium to colonize the urinary tract is due to the ability to synthesize protein appendages called fimbriae that help adhere to urinary epithelial cells and renal epithelial cells (6). The most important virulence factors of P.mirabilis are the production of urease, the production of haemolysin, the ability to adhere to epithelial cells and move in waves (swarming) by means of flagella, and the production of bacteriocin known as Protein (7). The study aims to determine the sensitivity of antibiotics to Proteus mirabilis bacteria isolated from the urinary tract at different pH levels (acidic, basic and neutral).

Antibiotics

They are biochemical substances produced as a secondary metabolite of some microorganisms. They may have a bacteriocidal or bacteriostatic effect. Antibiotics differ from each other in their mechanism of action in the bacterial cell (8). Antibiotic resistance is one of the biggest health problems worldwide, which prompts researchers to investigate new antibiotics to overcome resistant bacteria, as well as the need to distinguish between bacterial infections to avoid unnecessary use of antibiotics, which leads to the emergence of antibiotic resistance (9). The random and increasing use of antibiotics leads to the emergence of bacterial isolates resistant to many of the antibiotics used (10). There are two main types of antibiotic resistance, namely innate or natural resistance (Natural resisance) and acquired resistance (Acquired resistance). Natural resistance is of genetic origin as a result of bacteria possessing resistance genes carried on the chromosome or plasmid. As for acquired genetic resistance, it results from the acquisition of plasmids or a mutation in the resistance genes carried on the chromosome. Resistance may be attributed to transposable elements, and thus it turns into The sensitive strain of the antibiotic to the resistant (11). Plasmid-mediated resistance is one of the most important types of resistance because plasmids have the ability to move from one bacterial cell to another and cause the spread of resistance to many antibiotics (12). Bacteria resist antibiotics through several mechanisms depending on the type of antibiotic and the bacteria. These mechanisms include the presence of enzymes that mutate or degrade the antibiotic, the presence of alternative enzymes to the enzymes that are inhibited by antibiotics, modifying the target site of the antibiotic, reducing the penetration of the antibiotic into the cell, and possessing an active efflux mechanism that works to expel the antibiotic from the cell and prevent its accumulation inside the cell (13).

Beta-lactam antibiotics work by preventing the formation of peptide bridges that link amino acid units in the peptidoglycan layer within the bacterial cell wall, which makes the bacterial cell sensitive to osmotic pressure (14). Beta-lactam antibiotics include penicillins and cephalosporins, which contain a beta-lactam ring in their structure. Third-generation cephalosporins are the most effective generations against Proteobacteria, and they are used in urinary tract infections caused by P.mirabilis (15). Beta-lactam antibiotics also include the Carbapenem group, which has wide medical importance, including lmipenem and meromenem. The mechanism of action of aminoglycoside antibiotics is through preventing protein synthesis by binding to the 30S site of the ribosome, and inhibiting the initiation complex in the protein synthesis process, which leads to an error in reading mRNA and thus producing an ineffective protein (16). Tetracycline antibiotics, including (Methacclin, chlorotetracyclin, Tetracyclin, Oxytetracyclin), work by inhibiting protein synthesis by binding to the 30S part of the bacterial ribosome, thus preventing the binding of aminoacyl tRNA to the ribosome, which leads to stopping the synthesis of the polypeptide chain (17). The widespread use of tetracycline antibiotics leads to an increase in the percentage of resistant strains in most types of the intestinal family, which reduces the therapeutic importance of this antibiotic in treating bacterial infections.

Athromycin antibiotics, which are one of the macrolide antibiotics, work to stop protein synthesis by binding to the 50s part of the ribosome and interfering with the initiation complex, thus stopping the translation process and its effect is inhibitory to bacterial growth. Bacteria resist macrolide antibiotics by producing enzymes that inhibit the antibiotic. These enzymes work to hydrolyze the lactone ring of the macrolide nucleus, as well as produce phosphotransferase Type L enzymes that inhibit erythromycin antibiotics by adding a phosphate to the hydroxyl-2 group in the amino sugar of the antibiotic (18). Quinolines include a number of synthetic antibiotics, all of which are derived from Nalidxic acid, including Ciprofloxacin, Ofloxancin, and Norfloxacin, which are bactericidal antibiotics. Quinolines work to inhibit the activity of the bacterial DNA gyrus enzyme, which is responsible for unwinding the DNA strand during The process of replication and these enzymes also inhibit the enzyme Topoisomerasell, which leads to inhibiting the process of DNA replication and transcription (19).

Methods

2_1 Collection of pathological samples

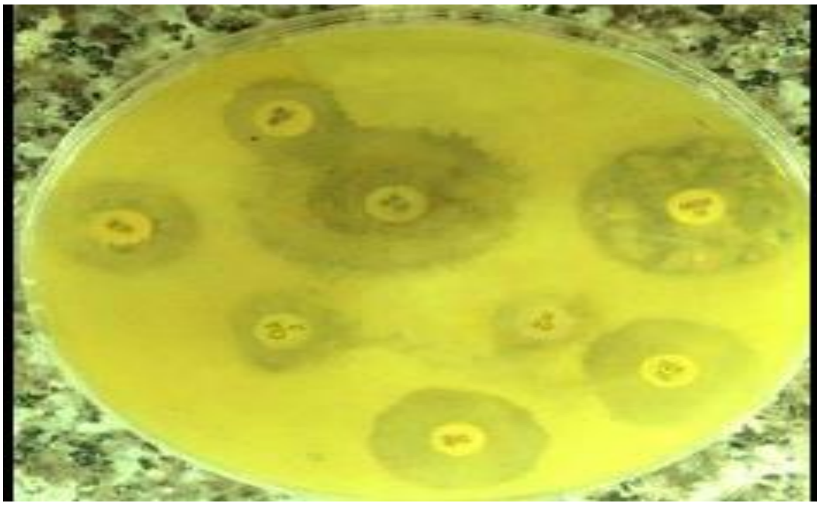
Urine samples were collected from patients suffering from urinary tract infections who had been previously diagnosed by specialist doctors and from different hospitals. Information related to the patient, such as age, gender, and other diseases, was recorded in a special form. For this purpose, sterile 50-ml bottles were used. The average urine sample was taken. It was cultured immediately after taking the sample for the purpose of diagnosis.

2_2 Urine Culture: Urine samples were cultured by the planning method (using a standard sterile culture carrier) on blood agar and MacConkey differential medium. The plates were incubated at 37°C for 18-24 hours. After that, the colonies were diagnosed morphologically based on their morphological characteristics, in terms of shape, size and color of those colonies. The isolates that gave the anthellae phenomenon as well as a fish-like odor on blood agar medium were selected. As for MacConkey medium, the pale color of the colonies was relied upon because they were non-lactose fermenter. The selected isolates were transferred to a new MacConkey medium.

2-3 Identification of isolates: Isolates were diagnosed morphologically and biochemically. The isolates were initially diagnosed based on the morphological characteristics of the colonies on MacConkey medium, and observing the anthellae phenomenon swarming on MacConkey medium. Blood.

2-4 Antibiotic susceptibility testing using the disc technique

Antibiotic susceptibility testing using the disc technique was performed on Mueller-Hinton agar medium based on what was mentioned in Atlas (1995) using antibiotic discs to perform the sensitivity test for the bacterial isolates under study. Bacterial cultures were prepared by transferring an isolated colony to 5 ml of nutrient broth medium and incubating at 37 ° C for 18-24 hours. The turbidity of the growth was compared with the turbidity of the standard turbidity constant solution. 0.1 ml of the above culture was spread in Mueller-Hinton agar medium using a sterile cotton swab. The plates were left to dry at room temperature for 10-15 minutes. The antibiotic discs were then transferred using sterile forceps to the plates at a rate of 5 discs per plate. The plates were incubated at 37 ° C for 24 hours. The results were read by observing the inhibition zones formed around the antibiotic discs and the results were interpreted according to the description mentioned in CLSI (2010).



Results and Discussion

For the purpose of studying the effect of hydrogen ion concentration on the response of isolates to antibiotics by disk diffusion method. 26 isolates belonging to the species P. mirabilis were selected, which were resistant to most antibiotics against 9 commonly used medical antibiotics and on three culture media with pH values of 4.5, 7 and 8.5, Table No(1) .

Table No. (1) shows the effect of pH on the resistance of P. mirabilis bacteria to antibiotics

PH=8.5		PH=7		PH= 4.5		Antibiotic
%	Number of resistant isolates	%	Number of resistant isolates	%	Number of resistant isolates	
7.6	2	23	6	38	10	Amikacin
11	3	23	6	26	7	Meronem
11	3	30	8	50	13	Ampnem
100	26	100	26	100	26	Ampicillin
84	22	84	22	84	22	Rifampin
88	15	61	16	57	23	Gentamicin
0	0	7.6	2	7.6	2	Ciprofloxacin
88	21	80	21	80	23	Trimethoprim

100	26	100	26	100	26	Tetracycline

1.In acidic medium (pH = 4.5), all isolates were resistant to ampicillin and tetracycline, while two isolates (7.6%) were resistant to ciprofloxacin, 7 isolates (26%) were resistant to meromenem, 10 isolates (38%) were resistant to amikacin, 13 isolates (50%) were resistant to ampfen, 22 isolates (84%) were resistant to rifampin, and 23 isolates (88%) were resistant to both gentamicin and trimethoprim. In neutral medium (pH = 7), all isolates (100%) were resistant to both ampicillin and tetracycline, two isolates (7.6%) were resistant to ciprofloxacin, and 6 isolates (23%) were resistant to lamicin and meromenem. 8 isolates (30%) were resistant to ampicillin, 16 isolates (61%) were resistant to gentamicin, 21 isolates (80%) were resistant to both trimethoprim and rifampin. 22 isolates (84%) were resistant to both trimethoprim and rifampin. In basic medium (pH = 8.5), all isolates were sensitive to ciprofloxacin. These results are consistent with what Zeiler (1985) reached, who indicated that the effectiveness of ciprofloxacin was increased in basic and neutral medium (pH = 8-7) than in acidic medium (pH < 7). Two isolates (6.7%) were resistant to amikacin and 15 isolates (57%) were resistant to gentamicin. These results are consistent with what was indicated by Fluit and his group (2001) who found an increase in the effectiveness of aminoglycosides (amikacin and gentamicin) in basic medium (20). The results in the current study showed an increase in the effectiveness of ciprofloxacin, amikacin, ampfenem and meropenem in basic medium than in acidic and neutral medium, which explains the effectiveness of these antibiotics in treating urinary tract infections caused by Proteus bacteria. The reason for the increased effectiveness of antibiotics in basic medium may be due to the fact that the process of transporting the antibiotic inside the cell may be faster in basic medium or perhaps the binding of the antibiotic to the target site is faster in basic medium compared to neutral medium.(21)

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Conclusion

1. There is a clear effect of pH on the effectiveness of antibiotics
2. Some antibiotics have high efficacy in neutral pH while their efficacy is weak in acidic pH media
3. Antibiotics effective in alkaline pH are the best for treating urinary tract infections resulting from infection with Proteus bacteria because they produce the enzyme urease which decomposes urea and changes urine to alkaline pH

Recommendations

1. Use antibiotics effective in alkaline medium to treat Proteus bacteria infections
2. Do not use antibiotics effective in alkaline medium to treat Proteus
3. Conduct broader studies on the effect of pH on the effect of pH on the action of antibiotics
4. Clothes must be sterilized and washed well.
5. We conclude that used clothes are not clean and unfit for use and cause many health problems.
6. Among the dangerous things that used clothes transmit are viruses, insects, parasites, their eggs, bacteria and fungi, which cause serious skin and respiratory diseases for humans.

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