Isolation and identification of Multidrug-Resistant (MDR) bacteria from ophthalmic patients in some hospitals in Basrah

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Abstract

104 samples of patients with eyes inflammation were collected from some hospitals and outpatient clinics in Basrah Province, 44 samples of males, at a percentage of 42%, and 60 samples of females, at a percentage of 58%, and they were of different ages. We found 74 developing bacteria samples (positive growth) at a percentage of 71%, and 30 samples with no bacterial growth at a percentage of 29%. 12 multi-drug resistant (MDR) bacterial samples were diagnosed, at a percentage of 16% of the total positive growth bacterial samples, represented by Staphylococcus aureus (50%), Escherichia coli (25%), Acinetobacter sp. (16.6%), and Proteus mirabilis (8.3%). The study revealed the resistance of all isolated samples of S. aureus bacteria to multiple antibiotics such as Trimethoprim, Methicillin, Cefoxitin, and Cefixime. Gram-negative bacterial isolated samples showed resistance to Cefixime, Trimethoprim, Aztreonam, Amoxicillin clavulanic acid, Gentamicin, and Tetracycline in varying proportions. The study also revealed the sensitivity of all Gram-positive bacterial samples to Ciprofloxacin, Chloramphenicol, Gentamicin, Vancomycin and Azithromycin antibiotics. It further indicated that some Gram-negative bacterial samples are sensitive to Piperacillin, Meropenem, Ciprofloxacin, and Chloramphenico.

Keywords: *multidrug-resistant, identification, ophthalmic.*

1. INTRODUCTION

The eye is one of the very sensitive and complex organs in the human body, and like any other organ, the eye can be attacked by some types of germs, such as bacteria, fungi, viruses, parasites, and others. The human immune system may be ready to fight them, but often the matter develops into inflammations. One of the most common types of inflammations is bacterial eye infection, which can affect any part of one or both eyes. Patients with an eye inflammation usually complain from pain, blurred vision, and redness in the eye. Conjunctivitis is the most common eye infection and rarely threatens vision. Keratitis and endophthalmitis are less common but pose a significant risk to vision. If the patient has a history of blurred vision, pain, sensitivity to light, corneal opacity or insufficiency, then this should be evaluated by a specialist doctor. Topical antibiotics should be avoided for eye inflammations unless the

patient is suffering from bacterial conjunctivitis (Watson et al., 2018).

Bacteria are the main contributors to eye inflammations worldwide. Eye inflammations, if left untreated, can lead to damage to eye structures with a potential for blindness and visual impairment. Bacteria are one of the leading causes of infectious diseases in the world, due to the resistance that bacterial species have developed to antibiotics (Kędziora et al., 2018). The World Health Organization (WHO) states that corneal diseases are the leading cause of vision loss after cataracts, and a cause of visual impairment and blindness worldwide (Khalil et al., 2020). WHO has published a list of bacterial families that pose the greatest threat to human health due to their resistance to numerous antibiotics. The list includes A. baumannii, E. coli, Proteus sp. and S. aureus (Mateo and Jiménez, 2022). The use of broad-spectrum antibiotic may lead to the development of resistance to commonly prescribed drugs leading to an increased risk of treatment failure (Diriba et al., 2020). There is a high global prevalence of Staphylococcus species resistant to antimicrobials among external eye pathogens. Resistance is increasing with decreased efficacy of many commonly used topical antimicrobials (Getahun et al., 2017).

2. Materials and Methods

2.1. Sample Collection: 104 samples from ophthalmic patients with bacterial inflammations were collected from some hospitals and outpatient clinics in Basrah Province, and then transferred to the laboratory and placed directly on the culturing media to be incubated for 24 hours at a temperature of 37° C.

2.2. Phenotypic Diagnosis: The phenotypic characteristics of the isolated colonies were studied after culturing the bacterial isolates and purifying them on culture media, and after cultivating them on MacConkey agar medium to distinguish between Gram-negative and Gram-positive bacteria and those that are lactose fermenting and non-lactose fermenting bacteria. Then, they were examined under an optical microscope to examine the shape, arrangement and colors of the cells according to their interaction with Gram stain, along with conducting a 3% KOH test to ensure that they are positive or negative to Gram.

2.3. Biochemical Tests: Some tests were conducted on Gram-positive and Gram-negative bacteria, such as the catalase test, the oxidase test, the motility test by hanging drop method, and the mannitol medium test.

2.4. Drug Sensitivity Test: The sensitivity of bacterial isolates to various antibiotics was tested based on CLSI (2020) according to the standard Kirby-Bauer disk diffusion method.

3. Results and Discussion:

In the current study, 104 samples of patients with eyes inflammation were collected; 44 samples of males, at a percentage of 42%, and 60 samples of females, at a percentage of 58%, and they were of different ages. We found 74 developing bacteria samples (positive growth) out of 104 (at a percentage of 71%), and 30 samples with no bacterial growth at a percentage of 29% as per table (1-2). This is consistent with the study of (Al-Douri and Maaroof, 2018). The results of the current study showed that the number of Gram-positive bacterial isolates is 54 (72.9%) and the number of Gram-negative bacterial isolates is 20 (27%) of the total developing bacterial isolates (positive growth) amounting to 74; which is consistent with (Khalil et al., 2017) and (Faisal and Al-Luaibi, 2022).

The results in Table (1-1) showed that the infection rate in females is 58%, which is greater than the rate in males (equaling 42%). These rates vary from one region to another depending on several factors, including health status, personal care, and living and economic

conditions (Sreeja et al., 2017). Another possible reason for the spread of infections in females may be due to the use of cosmetics such as lenses, eyeliner, mascara, or false eyelashes, or even different level of eye sensitivity to the ingredients in these substances (Dimri, 2022).

Table (1-1) showing the number of study samples by age group

Age Groups	Males	Females
<9	1 (2.3%)	7 (11.7 %)
10-19	4 (4.5%)	8 (13.3 %)
20-29	9 (20.5%)	6 (10 %)
30-39	10 (22.7%)	6 (10 %)
40-49	12 (27.3%)	14 (23.3%)
50-59	6 (13.6%)	14 (23.3%)
60-69	4 (9.1%)	12 (20%)
Total	44 (42%)	60 (58%)

Table (1-2) showing the number of study samples resistant to multiple antibiotics

Total Samples	Positive Growth Samples	Negative Growth Samples	Multidrug-resistant Samples
104	74 (71.2%)	30 (28.8%)	12 (16.2%)

In the current study, 12 multidrug resistance (MDR) bacterial samples were isolated, at a percentage of 16% of the total positive growth bacterial isolates collected from human eye infections in some hospitals and outpatient clinics in Basrah Province, while the remaining isolates varied between sensitive or resistant to only one or two antibiotic substances. The results of phenotypic diagnosis showed that the largest percentage of bacteria causing eye inflammations is Staphylococcus aureus at a percentage of 50%, followed by Escherichia coli at a percentage of 25%, Acinetobacter sp. at a percentage of 16.6%, and Proteus mirabilis by 8.3%, and these results are almost consistent with (Getahun et al., 2017) , (Teweldemedhin et al., 2017) and (Mahdi et al., 2021).

Table (1-3) Bacterial species resistant to multiple antibiotics and their percentage

S.	Species	Number of isolates	Percentage
1	Staphylococcus aureus	6	50 %
2	E.coli	3	25 %
3	Acinetobacter sp.	2	16.6 %
4	Proteus mirabilis	1	8.3 %
Total		12	

Table (1-4) Phenotypic Diagnosis of isolates resistant to multiple antibiotics

Isolate no.	Gram stain	3% KOH	MacConkey	lactose fermentation	cell shape	Diagnosis
1	_	+	+	_	Coccibacilli	*Proteus marbilis*
2	+	_	_	_	Cocci	*Staphylococcus aureus*
3	_	+	+	+	Rod	*Escherichia coli*
4	_	+	+	_	Coccibacilli	*Acinetobacter* sp.
5	_	+	+	+	Rod	*Escherichia coli*
6	_	+	+	_	Coccibacilli	*Acinetobacter* sp.
7	+	_	_	_	Cocci	*Staphylococcus aureus*
8	+	_	_	_	Cocci	*Staphylococcus aureus*
9	+	_	_	_	Cocci	*Staphylococcus aureus*
10	+	_	_	_	Cocci	*Staphylococcus aureus*

11	_	+	+	+	Rod	*Escherichia coli*
12	+	_	_	_	Cocci	*Staphylococcus aureus*

Table (1-5) Biochemical tests for bacteria resistant to multiple antibiotics

S	Bacterial Strains	Catalase Test	Oxidase Test	Motility Test	KOH (3%)	Lactose ferment
1	*Staphylococcus aureus*	+			–	–
2	*Escherichia coli*		–	+	+	+
3	*Acinetobacter* sp.		–	–	+	–
4	*Proteus marbilis*		–	+	+	–

Antibiotics Sensitivity Test Results for Isolated Bacteria

The results of the study showed that all isolates of Staphylococcus aureus were characterized by their resistance to multiple antibiotics such as Trimethoprim, Methicillin, Cefoxitin, and Cefixime, which is consistent with (Getahun et al., 2017), as S. aureus is an important pathogen, and the bacteria's production of biofilms is an important factor in increasing their multidrug resistance (Senobar Tahaei et al., 2021). The cause of resistance may be attributed to the presence of efflux pumps that help them flush antibiotics out of the bacterial cell (Papkou et al., 2020).

The current study also showed the resistance of the Gram-negative bacterial isolates to the Cefixime, Trimethoprim, Aztreonam, Amoxicillin clavulanic acid, Gentamicin and Tetracycline antibiotics in varying strengths. The resistance may be due to several reasons, including the presence of efflux pumps in bacteria, as well as their production of β-lactamases enzymes, which dismantle the β-lactams ring and inhibit the action of antibiotics belonging to the penicillins and cephalosporins (Paltansing, 2015).

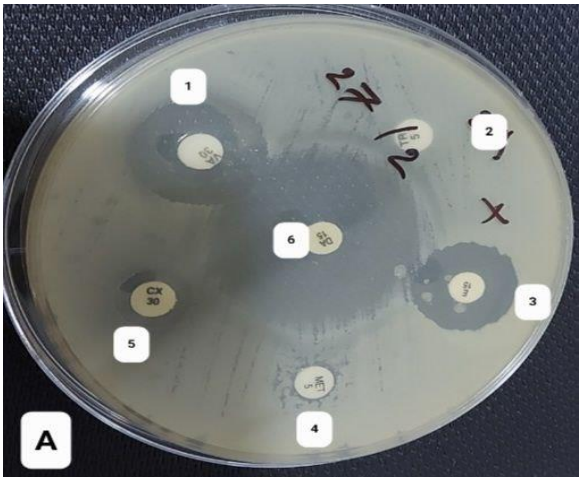
The results also showed the sensitivity of all Gram-positive isolates to the Ciprofloxacin, Chloramphenicol, Gentamicin, Vancomycin and Azithrthromycin antibiotics, and this is consistent with (Getahun et al., 2017), while the results also indicated that some Gram-negative bacterial isolates are sensitive to the Piperacillin, Meropenem, Ciprofloxacin and Chloramphenicol antibiotics, which is consistent with (Khalil et al., 2017). The results further showed that all Gram-negative and Gram-positive bacterial isolates are 100% sensitive to the Ciprofloxacin antibiotic, which is consistent with (Abd Al Wahid and Abd Al-Abbas, 2019) and (Alshamahi et al., 2020). The reason for this difference in the sensitivity or resistance of bacterial isolates can be explained by the extent to which these isolates are exposed to factors that lead to the emergence of resistance, including the indiscriminate use of antibiotics for long periods without following medical instructions (Al-Tamimi et al., 2022).

Table (1-6) Drug sensitivity test for multidrug resistant isolates

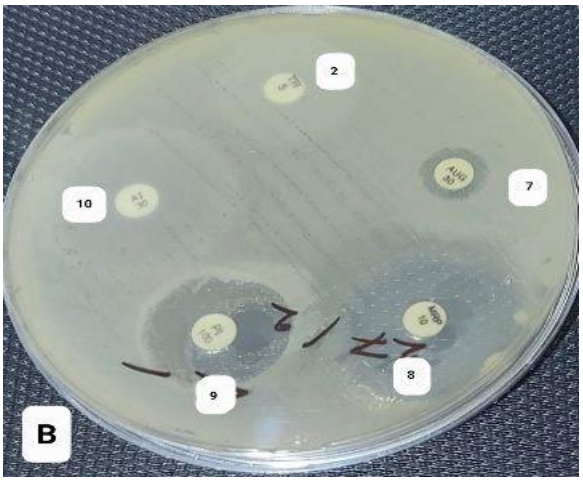
Antibiotic / NO.	CFM5	TR5	TE30	AT15	CN10	CIP10	C10	E15	CX30	VA30	DA15	MET5	AT30	MRP10	PI100	AUG 30
1	R	R	S	S	S	S	S	S	13	I	S	R				
2	R	R	I	S	S	S	S						R	S	S	S
3	R	R	I	I	S	S	I	I					R	S	S	10
4	R		R	R	I	S	I						S	S	S	I
5	R	R	R	S	S	S	S						R	S	I	S
6	R		I	S	R	S	I						R	S	I	S
7	R					S			R			R				
8	R					S			R			R				
9	R					S			R			R				
10	R					S			R			R				
11	R	R	R			S							R	S	I	S
12	R	R				S			R			R				

R= Resistant, I=Intermediate, S= Sensitive

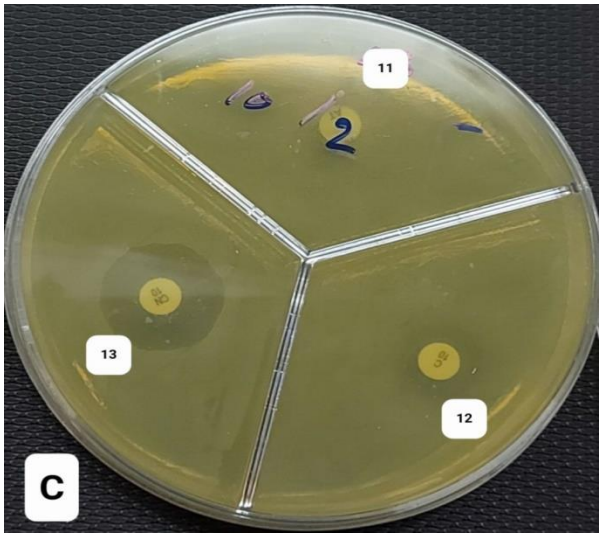
Figure 1 shows drug sensitivity testing on Mueller-Hinton Agar plates



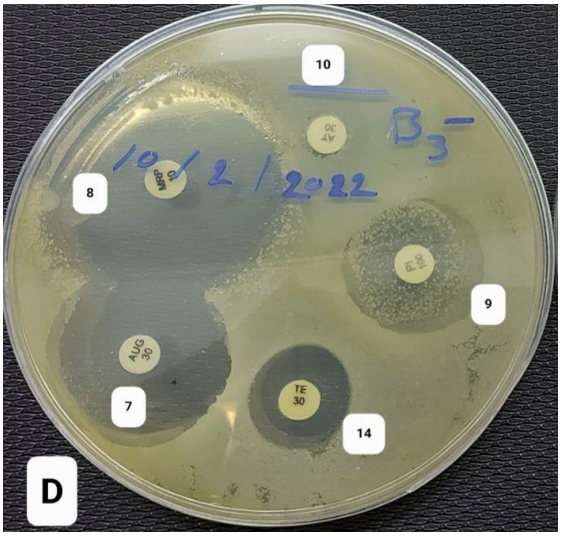
A- Staphylococcus aureus



B- Escherichia coli



C- Acinetobacter sp.



D- Proteus marbilis

1= Vancomycin 30mg, 2= Trimethoprim 5mg, 3=Erythromycin15mg, 4= Methicillin 5mg, 5= = Cefoxitin 30mg, 6= Clindamycin30mg, 7= Amoxacillin clavulanic 30mg, 8= Meropenem10mg, 9= Piperacillin 100mg, 10= Aztreonam 30mg, 11= Azithromycin 15mg, 12= Chloramphenicol 10mg, 13= Gentamicin 10mg, 14= Tetraccyclin 30mg

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