RESEARCH
O&G Forum 2024; 34 – 3s: 1976-1989

DETECTION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA FROM IRAQI WOMEN INFECTED WITH POLYCYSTIC OVARY SYNDROME

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Abstract

Polycystic ovary syndrome is an endocrine disorder affecting women of reproductive age with a worldwide prevalence of approximately 8-13%. In patients with PCOS, numerous small cysts (fluid-filled sacs) form in the ovaries. Typical clinical features include abnormally high levels of androgens, irregular menstrual cycles, hirsutism, acne, obesity, and infertility. PCOS pathogenesis remains elusive, but emerging research highlights the role of human microbiome in its occurrence and progression . the total number of samples 60 vaginal swabs. Vaginal swab samples were collected from women with polycystic ovary syndrome. During the period between 10 November 2023 and 20 February 2024 in Mosul City hospitals (Al-Khansa Teaching Hospital and Al-Salam Teaching Hospital), included different age groups ranging from 18 to 45 years. The aim of this study was to detect gram positive and gram negative bacteria in high vaginal swab from polycystic ovary syndrome. The result showed 15 were Gram-positive bacterial isolates (25%) and 45 were Gram-negative bacterial isolates (75%) . The result showed that the vaginal infection more frequent in age between (27-35) years old. According to age groupings, the age group(27-35)years old had the highest prevalence of vaginal infection with bacteria among patients (26; 43.34 %), 4 patients (6.67%)**,** for Gram-positive bacterial. Conclusion there is microbial diversity in the vaginal microbiota. Incidence with Gram-negative bacteria were predominant more than Gram-positive bacteria in PCOS patients especially Staphylococcus. spp and E. coli in PCOS women.

Keywords: PCOS, Gram-positive , Gram-negative, HVS.16Sr RNA.

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting women of reproductive age with a worldwide prevalence of approximately 8–13%**(**Zheng *et al*., 2024**)**. In patients with PCOS, numerous small cysts (fluid-filled sacs) form in the ovaries. Typical clinical features include abnormally high levels of androgens, irregular menstrual cycles, hirsutism, acne, obesity, and infertility(Jobira *et al*., 2020). PCOS pathogenesis remains elusive, but emerging research highlights the role of human microbiome in its occurrence and progression (Duan *et al*., 2021). Studies have shown that PCOS is associated with dysbiosis of the gut microbiota (Jobira *et al*., 2020;Sun *et al*., 2023) A few studies indicate that changes in the microbiome might have a role in the development of PCOS (Giampaolino *et al*., 2021). Despite recent research showing a link between disruptions in the gut microbiome and the development of metabolic diseases. But the correlation between different vaginal microbiomes and the etiology and pathogenesis of PCOS is poorly understood (Hong *et al* ., 2020). Numerous typical microbial and fungal groups, with lactobacilli as the predominant genus, colonize the vagina of a healthy female. These groups are categorized as beneficial microbes and opportunistic pathogens (Chen et al., 2017). Patients with PCOS have reduced diversity and altered composition of the gut microbiota, such as a decrease in Lactobacillus and Bifidobacterium, and an impaired intestinal mucosal barrier, compared to those without any health problems. The alterations in the gut microbiota have been linked to levels of inflammation and insulin resistance, by altering the stability of the intestinal. In women of reproductive age, a healthy vaginal microbiome typically exhibits a low pH (4.2 to 5.0) in different ethnic groups, with a limited presence of dominant facultative anaerobic bacteria, such as Lactobacillus crispatus and L. inner, which help maintain an acidic environment and produce H2O2 as

a defense against pathogen invasion (Hong *et al* ., 2020; Wright *et al*., 2021). The main variables influencing the vaginal microbiota include the effects of estrogen and progesterone on vaginal epithelial cells, PH, sexual activity, menstruation, and antibiotic use (Smith et al., 2017). As vaginal bacteria are an essential component of the reproductive tract's milieu, the body gains from their homeostasis.(Moosa *et al*., 2020). There is mounting evidence that a woman's vaginal microbiota composition can have a major impact on her sexual and reproductive health, including her risk of HIV infection and other STDs, as well as unfavourable delivery outcomes like miscarriage and premature delivery (Elovitz et al.,2019; Mohamed et al., 2020). Also, all Gram-negative bacteria, like *E. coli*, have the most important structure in their cell walls, stimulate the immune system, and produce many cytokines such as TNF-α and IL-6 (Torcia et al.,2019). Along with vaginal bacteria, neutrophils, macrophages, classical dendritic cells, Langerhans cells, NK cells, T and B lymphocytes, and other innate and adaptive immune cells, the stratified squamous epithelial cells that cover the mucus layer are a part of the vaginal ecosystem (Tu *et al*., 2020).All Gram-negative bacteria, like E. coli, have the most important structure in their cell wall, stimulate the immune system, and produce many cytokines such as TNF-α and IL-6 (Torcia et al.,2019).

Material and Methods

This study was conducted in Nineveh Governorate, the total number of samples 60 vaginal swabs. Vaginal swab samples were collected from women with polycystic ovary syndrome. During the period between 10 November 2023 and 20 February 2024 in Mosul City hospitals (Al-Khansa Teaching Hospital and Al-Salam Teaching Hospital), included different age groups ranging from 18 to 45 years. Specialised gynaecologists collected vaginal swab samples from the lateral posterior vaginal fornix using Amies transport medium. After that, the samples were sent directly to the university of Mosul's microbiology lab while following to standard laboratory procedures. Freshly prepared media (Nutrient agar, MacConkey agar, Blood agar, Chocolate agar) were prepared for isolation and identification of bacteria; the medium was inoculated with the swab and incubated aerobically and anaerobically(CO_2) at 37◦ C for 24 hours. All isolates were purified by sub-culturing and then Gram stain was applied for differentiation between isolated bacteria under oil immersion (100X).The isolated and purified bacteria was identified according to the bacterial morphology and colony characters. then, all isolated bacteria were identified by the VITEK-2 Compact bioMérieux, VITEK-2 cards were inoculated following the manufacturer's instructions and the freshly bacterial culture were used which have less than 18hrs age, also we use extract bacterial genome by using (Geneaid bacterial extraction kit) then sent 16sr sequence for some variable isolate and then submitted in NCBI .

Identification By Using Vitik-2Compact System

(**60**) bacterial isolates from PCOS patients were examined using Vitek-2compact system at the university of Mosul's microbiology lab. The identified of Gram-positive and Gram-negative bacterial isolates were confirmed with the automated Vitek-2compact system by using GPB-592 Kit (ID) and GNB-222Kit(ID) cards). The cards have bar codes that contain information on product type, lot number and expiration date. The biochemical interactions between both the media in the VITEK-2compact system identification cards and the bacterial specimens suspended in their solutions were measuring various metabolic activities (64) tests , required up to (6) hours to identify (115) Gram-positive taxa and (135) Gramnegative taxa. All the following steps are prepared according to the manufacturer's instructions (BioMerieux, France).

Identification By 16S rRNA Gene Sequencing

Colonies that were that not detected by Vitik-2Compact System ,were further identified by 16S rRNA gene sequencing. PCR products were run on 1% agarose and stained with Midori Green Advance DNA stain (Germany). Purified PCR products were sent for sequencing at Psomagen company (USA).

PCR And Genomic DNA Isolation

DNA was extracted from bacterial isolate using Geneaid DNA extraction kit following the steps recommended by the company. Primers used for amplification and estimated product size for 16rs in table (1). PCR of *16rs* genes was performed in 25µl reaction using Promega master mix following recommended conditions and using the protocols listed in tables (2), respectively. Amplification of 16S rRNA gene was conducted as shown in table (3).

Table (1): List of primers used in this study.

Primer name	Sequence (5' - 3')	Product size (bp)	Reference
27F **1522R**	AGAGTTTGATCMTGGCTCAG AAGGAGGTGATCCARCCGCA	1500	(Nagara *et al*., 2017)

Table (2): Components Of The Polymerase Chain Reaction Mixture And Their Sizes

NO.	Components	Volume in microliters
1	DNA Template	3 µl
2	Primer Forward	2 µl
3	Primer Reverse	2 µl
4	Nuclease free water	6 µl
5	Taq Green master mix	12 µl
6	Final volum	25 µl

Table (3) : Conditions for amplifying the 16S rRNA gene from all isolates

NO.	Main steps	No. of cycles	Temperature C°	Time
1	**Initial denaturation**	**1**	**95**	3 minutes
2	**Denaturation**	**30**	**95**	30 seconds
3	**Annealing**		**54**	30 seconds
4	**Extention**		**72**	30 seconds
5	**Final Extention**	**1**	**72**	3 minutes

Results

Sixty vaginal swab collected from PCOS women that revealed, of the 60 PCOS patients, 15 were Gram-positive bacterial isolates (25%) and 45 were Gram-negative bacterial isolates (75%) shown in table (4). The result showed that the vaginal infection more frequent in age between (27-35) years old. According to age groupings, the age group(27-35)years old had the highest prevalence of vaginal infection with bacteria among patients (26; 43.34 %), 4 patients (6.67%), for Gram-positive bacterial. The results in table (5) showed that most PCOS women infected with Staphylococcus. spp (No.=11; 18.33%) and E. coli (No.= 22;36.67%%) were the most common isolates PCOS women.The bacterial isolate that submitted in NCBI were; *Stutzerimonas stutzeri* strain WAHI, *Klebsiella pneumonia* strain WAHI1, *Klebsiella pneumonia* strain WAHI24, *Klebsiella pneumonia* strain WAHI94, *Klebsiella pneumonia* strain WAHI15, *Klebsiella pneumonia* strain HIWA, *Proteus mirabiliss* strain HIWA1, *Escherichia coli* strain HIWA24 , *Klebsiella pneumonia* strain HIWA94 And their bands as shown in figure (1).

Table (4): Distribution of gram positive and gram negative bacterial isolate according to age and their percentage.

Ages (years)	Bacterial growth				Total	
	G + ve		G -ve			
	NO.	%	NO.	%	NO.	%
18-26	5	8.33%	19	31.6%	24	39.9%
27-35	4	6.67%	22	36.67%	26	43.3%
36-45	6	10%	4	6.67%	10	16.6%
Total	15	25%	45	75%	60	100%

No. means count of isolate ,Gr + means gram positive bacteria , Gr - means gram negative bacteria

TABLE (5): Frequency of bacterial identified isolated from HVS specimens of Iraqi women with PCOS.

Types of bacterial isolates	PCOS women with HVS infection	
	No.	%

Staphylococcus aureus	**5**	**8.33%**
Staphylococcus haemolyticus	**6**	**10%**
Streptococcus thoraltensis	**1**	**1.66%**
Enerococcus faecalis	**3**	**5%**
Escherichia coli	**22**	**36.67%**
Klebsiella pneumoniae	**20**	**33.33**
Proteus mirabilis	**2**	**3.33**
Pseudomonas stutzeri	**1**	**1.67**
Total	**60**	**100%**

PCOS: polycystic ovary syndrome, HVS: high vaginal swab , No: means count of isolate

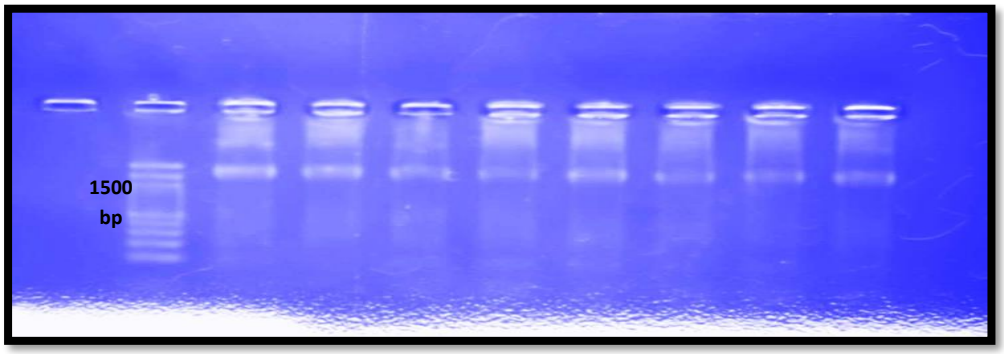

Figure (1): Agarose gel electrophoresis patterns show amplified PCR products of 16Sr RNA

AST And MICs Profile For Gram-Positive Bacteria:

Table (6):AST And MICs Profile For *S. aureus* , CoNS And *E. faecalis*

CoNS: Coagulase-Negative Staphylococcus

N	Antibiotics		*S. aureus (2)*			CoNS (4)			*E. faecalis (2)*		
			S (100	R (100	MIC	S (100%)	R (100%)	MIC	S (100	R (100	MIC
1	Benzylpenicillin	P	1(50)	1(50	>=	1(25)	3(75)	<=	2(100	0(0)	<=0.5
2	Clindamycin	CM	1(50)	1(50	<=0.	0(0)	4(100)	2	0(0)	2(100	8
3	Erythromycin	ER	1(50)	1(50	>= 8	0(0)	4(100)	>= 8	0(0)	2(100	<= 8
4	Gentamicin	GM	2(100	0(0)	<=	1(25)	3(75)	<=	1(50)	1(50)	<= 0.5
5	Ciprofloxacin	CIP	0(0)	0(0)	0	2(50)	2(50)	1	2(100	0(0)	1
6	Oxacillin	OX	2(100	0(0)	>= 4	0(0)	4(100)	>= 4	0(0)	2(100	<= 4
7	Rifampicin	RA	1(50)	1(50	<=	0(0)	4(100)	>=32	0(0)	2(100	>= 32
8	Teicoplanin		2(100	0(0)	<=	1(25)	3(75)	1	2(100	0(0)	<=
9	Tetracycline	TE	1(50)	1(50	<= 1	0(0)	4(100)	16	0(0)	2(100	>= 16
10	Tigecycline	TI	2(100	0(0)	<=0.	4(100)	0(0)	0.5	2(100	0(0)	<=
11	Trimethoprim/ Sulfamethoxazole	SX	2(100	0(0)	<=	3(75)	1(25)	<= 10	0(0)	2(100	<= 10
12	Vancomycin	VA	2(100	0(0)	1	3(75)	1(25)	4	1(50)	1(50)	8

Table(7):AST And MICs Profile For *E. coli* And *K. pneumonia*

No.	Antibiotics		*E. coli* (4)			*K. pneumonia* (2)		
			S (100%)	**R(100%)**	**MIC**	**S (100%)**	**R (100%)**	MIC
1	**Amikacin**	**AK**	4(100)	0(0)	<= 2	2(100)	0(0)	<= 2
2	**Cefepime**	**FED**	2(50)	2(50)	2	0(0)	2(100)	>= 64
3	**Ceftazidime**	**CTX**	0(0)	4(100)	16	1(50)	1(50)	>= 64
4	**Ciprofloxacin**	**CIP**	2(50)	2(50)	<= 0.25	1(50)	1(50)	>= 4
5	**Gentamicin**	**GM**	3(75)	1(25)	<= 1	2(100)	0(0)	<= 1
6	**Imipenem**	**IPM**	4(100)	0(0)	<= 0.25	2(100)	0(0)	<= 0.25
7	**Meropenem**	**MEM**	4(100)	0(0)	<= 0.25	2(100)	0(0)	1
8	**Piperacillin**	**P**	0(0)	4(100)	>= 128	0(0)	2(100)	>= 128
9	**Piperacillin/Tazobactam**	**TZP**	0(0)	4(100)	>= 128	0(0)	2(100)	>= 128
10	**Ticarcillin**	**TIC**	0(0)	4(100)	>= 128	0(0)	2(100)	>= 128
11	**Ticarcillin/Clavulanic Acid**	**TIC**	0(0)	4(100)	<= 8	1(50)	1(50)	>= 128
12	**Trimethoprim/ Sulfamethoxazole**	**SXT**	3(75)	1(25)	<= 20	1(50)	1(50)	64
13	**Tobramycin**	TOB	2(50)	2(50)	<= 1	0(0)	2(100)	4

Table (8): AST And MICs Profile For *P. stutzeri* And *Proteus. mirabilis*

N O	**Antibiotics**		***P. stutzeri (1)***			***Proteus. mirabilis(2)***		
			S (100%)	**R(100%)**	**MIC**	**S (100%)**	**R (100%)**	**MIC**
1	**Amikacin**	**AK**	**1(100)**	**0(0)**	**4**	**2(100)**	**0(0)**	**<= 2**
2	**Cefepime**	**FED**	**1(100)**	**0(0)**	**16**	**2(100)**	**0(0)**	**8**
3	**Ceftazidime**	**CTX**	**1(100)**	**0(0)**	**8**	**2(100)**	**0(0)**	**8**
4	**Ciprofloxacin**	**CIP**	**0(0)**	**1(100)**	**>= 4**	**0(0)**	**2(100)**	**>= 4**
5	**Gentamicin**	**GM**	**1(100)**	**0(0)**	**<= 1**	**1(50)**	**1(50)**	**4**
6	**Imipenem**	**IPM**	**1(100)**	**0(0)**	**1**	**0(0)**	**2(100)**	**8**
7	**Meropenem**	**MEM**	**1(100)**	**0(0)**	**1**	**2(100)**	**0(0)**	**1**
8	**Piperacillin**	**P**	**0(0)**	**1(100)**	**>= 64**	**0(0)**	**2(100)**	**>= 128**
9	**Piperacillin/Tazobactam**	**TZP**	**0(0)**	**1(100)**	**>= 128**	**2(100)**	**0(0)**	**8**
10	**Ticarcillin**	**TIC**	**0(0)**	**1(100)**	**>= 128**	**0(0)**	**2(100)**	**>= 128**
11	**Ticarcillin/Clavulanic Acid**	**TIC**	**1(100)**	**0(0)**	**<= 32**	**0(0)**	**2(100)**	**>= 128**
12	**Trimethoprim/ Sulfamethoxazole**	**SXT**	**0(0)**	**1(100)**	**>= 320**	**0(0)**	**2(100)**	**>= 320**
13	**Tobramycin**	**TOB**	**0(0)**	**1(100)**	**8**	**0(0)**	**2(100)**	**8**

Discussion

The results in table (5) showed that most PCOS women infected with Staphylococcus. spp (No.=11; 18.33%) and E. coli (No.= 22;36.67%%) were the most common isolates PCOS women. According to the results of our investigation, bacterial vaginal infections were present in every woman with polycystic ovarian syndrome. In line with other recent studies, the current investigation of PCOS patients' vaginal reveals that these patients have more diverse microbiomes (Hong *et al* ., 2020; Ahmed *et al*., 2021). The findings of this investigation bear a similarity to those of a study conducted in 2021 (Al-Wandawy et al., 2020) in which the same species of bacteria (*Staphylococcus auras*, *Enerococcus faecalis*, *Escherichia coli*, and *Klebsiella pneumoniae*) were found, along with some differences; however, some species of bacteria such as (*Staphylococcus haemolyticus*) were absent. Additionally, our study's results did not support the

findings of that study, which suggested that the PCOS group in that study had a suitable environment for the growth and multiplication of Candida. spp due to insulin resistance or glucose tolerance brought on by PCOS pathology. In the current study didn't detect Neisseria gonorrhea or Chlamydia trachomatis, this result not agreed with (3) study who found abundant Chlamydia trachomatis in the PCOS group, this differences in the result may be explained by the variations in the cultural approaches used, the social and ethnic diversity of the two study communities, and the sample size could all contribute to the differences in the results. In this study we detected many other microbiotas e.g. (*streptococcus thoraltensis, Proteus mirabilis, Pseudomonas stutzeri*), All members of the genus Pseudomonas have ability to grow on MacConkey agar medium, does not ferment lactose, these results reflect the huge variation of the vaginal inhabitants and disruption of vaginal flora in PCOS group. There was some of this isolates detected by 16rs technique and recorded in NCBI.

Percentage Of Gram-Positive Bacterial Species:

Gram-positive isolates were represented by *Staphylococcus aureus* 5/15(%) and *Staphylococcus haemolyticus* also accounted for 6/15(%), they were predominant isolate followed by *Enerococcus faecalis* 3/15(%) and *streptococcus thoraltensis* 1/15(%). Our results table (5) indicated the presence of G-positive bacterial species which were also obtained by other investigator with a local study in Baghdad ,Iraq (Al-Wandawy *et al*., 2020). Also, in our current study, we were able to diagnosis rare case with *streptococcus thoraltensis* in vaginal infection by using Vitek 2system for patient women visiting Gynecology Consultation Unit in Mosul City Hospitals/Iraq, we notice rare cases reported by locally previous studies (de Jong *et al.*, 2022)
Staphylococcus aureus The reason that makes *S.aureus* to be more invasive among Gram-positive organisms and an important predominance pathogen that leads to vaginal infection can be ascribed to the virulence factors, since *S. aureus* has four proteins having important roles in pathogenicity by allowing bacteria to avoid host defenses and by acting as adhesions, which have been characterized: protein A (immunoglobulin binding protein), collagen binding protein, fibronectin binding proteins, and the fibrinogen binding protein (clumping factor),(Nandhini *et al*., 2022). Predominance of *S. aureus* also may be related to its ability to resist many antibiotics especially methicillin (MRSA) which facilitated its widespread to environment (Weber *et al*.,2019).

Coagulase negative staphylococci (CoNS) appear to be the major pathogen worldwide and associated with significant UTI and HVI, and it is considered the most common organisms associated with UTI and HVI. In this study, CoNS represented the second pathogen among Gram-positive isolates . The reason of high rate of CoNS isolates may be related to the use of broad spectrum antibiotics and to the role of specific adhesion and slime produced by CoNS, since slim can reduce the immune response and opsonophagocytosis .

In humans, *Enterococcus. spp* are part of intestinal microbiota; they can be found in smaller proportions in secretions (oropharyngeal and vaginal)and on skin. The wide spectrum of clinical infections caused by *E. faecalis and E. faecium* can include urinary tract, endocarditis, and bacteremia (Daca *et al*., 2024). *Enerococcus faecalis* is a gram-positive, facultatively anaerobic bacteria. They are considered quite resilient strains because e.g., they can grow in a wide range of temperatures and pH. The successful culture can be maintained in temperatures between 5 and 50 °C and pH as high as 9.6 . The temperature resistance is linked to the high content of lipids and fatty acids in the membrane. The ability to survive in high pH and the presence of bile salts and enzymes allows them to colonize the small as well as the large intestine(Lin et al.,2021). Among *Enterococcus* species, *Enterococcus faecalis* is the primary species responsible for human enterococcal infections .Treatment of *E. faecalis* infections has become increasingly difficult because of the emergence of *E. faecalis* strains that are resistant to numerous clinically used antibiotics (Jennings *et al*.,2024). Enterococci are common commensal bacteria that colonize the gastrointestinal tracts of most mammals, including humans. Importantly, these bacteria are one of the leading causes of nosocomial infections(Ibraheem *et al*., 2022).

Percentage Of Gram-Negative Bacterial Species

Gram-negative bacterial isolate were represented by K. pneumonia 20/45(%), E. coli 22/45(%), *Pseudomonas stutzeri* 1/45(%) and Proteus mirabilis 2/45(%) . The results indicated the presence of Gram-negative bacteria which were also obtained by other previous local studies(Al-Wandawy *et al*., 2020) vaginal infection in PCOS women *Escherichia coli* was the most common pathogen present in UTI and HVI in PCOS women that agreeing with(Al-Musawy *et al.*, 2018,Al-Wandawy *et al.*, 2020)respectively who confirm in their studies that the E. coli was the most prevalent bacteria, possible E. coli infections in females because they are more susceptible to UTIs and bacterial vaginal infection than males due to anatomical differences, such as a shorter urethra and perineal contamination of the urinary tract with fecal microorganisms(Naqid *et al*., 2020).Since perianal and vaginal commensals are the most common sources of uropathogens, a focus on personal hygiene, especially in females, may be important to minimize uropathogenic infections(Vagarali *et al*., 2008, Subashchandrabose *et al*., 2017). *E.coli* display polymeric adhesive fibers termed "pili" or "fimbriae" that facilitate the initial attachment to epithelial cells and subsequent successful colonization of the host. E.coli secretes a range of virulence components at the host-pathogen interface, via membrane vesicle trafficking as bacterial outer membrane vesicles(OMVs) for invasion, endotoxic shock and other cell-cell communications .E. coli's Fitness and ability to persist in

surroundings are improved by the ability to quickness the metabolism of nutrient-rich conditions (Mendes *et al*., 2023).may also penetrate and reproduce within the bladder, generating biofilm-like intracellular bacterial communities (IBCs) and creating intracellular reservoirs for UTIs . *Klebsiella pneumoniae* is a gram-negative, encapsulated, non-motile bacterium. K. pneumoniae strains colonize the mucosal surfaces, including the gastrointestinal tract and oropharynx and from these sites, they can gain access to other tissues and cause life-threatening infections(Podschun *et al*., 1998). its name comes from the German bacteriologist Edwin Klebs, who first identified it in the 19th century(Geng *et al*.,2024). exhibits the ability to form biofilms as a means of adapting to its adverse surroundings(Choby *et al*., 2020). *Klebsiella pneumoniae i*s considered to be the most common cause of hospital-acquired infections (HAIs), accounting for 10% of all nosocomial infections worldwide (Yang *et al.,* 2023). Immunocompromised patients are at higher risk of HAIs, comprising 8% to 12% of hospitalized patients, particularly ventilator-associated pneumonia(Zhu *et al*., 2023). This can result in life-threatening illnesses with mortality rates ranging from 50% to 100% (Al-Busaidi *et al.,* 2024;Janda *et al*., 2021).*Proteus mirabilis* is a species of Gram-negative bacteria belonging to genus *Proteus* of Enterobacterales (Schaffer *et al*., 2017), and is widely distributed in the natural environment and intestines of humans and animals. *P. mirabilis* is one of the most frequently opportunistic pathogen causing nosocomial infection, especially causing urinary tract infection (Gong *et al*., 2019). In addition, *P. mirabilis* infections can also cause bacteremia and the formation of urinary stones. It is worth noting that some food poisoning cases associated with *P. mirabilis* had been reported in China, as well as other countries (Choby *et al*., 2020). is a pathogenic, Gram-negative, rod-shaped bacterium ,Swarming motility, urease production, biofilm formation, and the properties of its lipopolysaccharide (LPS) are all factors that contribute to the virulence of this bacterium. That swarms across solid surfaces, which often leads to catheter-associated urinary tract infections(speculum). The hindered eradication of *P. mirabilis* results in recurrent urinary tract infections(Tropea *et al*., 2007). Opportunistic pathogenic bacteria that are leading causes of foodborne illnesses and deaths is a serious threat to global public health (Lapage *et al*., 1968).

Pseudomonas stutzeri is common name *stutzerimonas stutzeri*. rod shaped bacteria approximately1 to 3 μm long and 0.5 μm in width, and have a solitary polar flagellum.11 Phenotypic characteristics of the class incorporate a negative Gram stain, positive catalase and oxidase tests, and a stringently respiratory metabolism(Tabassum *et al*., 2022). Is a nonfluorescent denitrifying bacterium broadly dispersed in the environment,that has been accounted for as a causative specialist of certain diseases which has been isolated as an opportunistic pathogen from human. It has been accounted as a cause of pneumonia, meningitis, visual disease, osteomyelitis and joint diseases. Thus, this species could be considered an opportunistic but rare pathogen.In addition, possess an extensive variety of resistance mechanisms against diverse group of antibiotics(Bisharat *et al*., 2012). For a about 15-year time span after 1956, a few reports depicted the isolation of *Pseudomonas stutzeri* from clinical and neurotic samples(Jennings *et al*.,2024). In any case, there was no unmistakable relationship of this species with pathogenesis cycle(Ibraheem *et al.,* 2022). However later, a couple of instances of *Pseudomonas stutzeri* associated diseases have been accounted for in relationship with bacteremia/septicemia, bone/joint infection, osteomyelitis, joint inflammation, endocarditis, endophthalmitis, meningitis, pneumonia or potentially empyema, ecthyma gangrenosum (Kose *et al*., 2004;Livermore *et al*., 2007).

Identification By Using VITEK 2system

Final identification for Gram-positive and Gram-negative bacterial isolates 60/60(100%) from PCOS women were dependent on the VITEK 2system exhibited excellent accuracy for the detection of bacterial isolates. After isolation and identification, in this study, the bacterial isolates were distributed into (60) isolates, 45/60(75%) belonging to Gram-negative bacteria were predominant .

AST And MICs Profile

(8) isolates under study were subjected to an antibiotic susceptibility test and minimum inhibitory concentrations in order to find out the prevalence of antibiotic resistance among these isolates. In this test, different groups of antibiotics were used such as the group of Penicillins, β-lactam-β-lactamase inhibitor, Carbapenems, Cephalosporins, Glycopeptides, Aminoglycosides, Sulfonamides, Fluiroquinoline, and other group by using VITEK 2compact system for infection control, and restrict therapeutic failures that may be triggered by the traditional methods (Ahmed *et al*.,2021). The etiology of bacteria that cause vaginal infection, urinary tract infection(UTIs), hospitalization-acquired infection and community-acquired infection, as well as their resistance to antimicrobials, have changed over time and differs between countries(Magliano *et al*., 2012; Ahmed *et al*.,2022).

AST And MICs Profile For Gram-Positive Bacteria

Most Gram-positive bacterial isolates were resistant to most antibiotics, including Benzylpenicillin, Clindamycin, Erythromycin, Gentamicin, Ciprofloxacin, Oxacillin, Teicoplanin, Tetracycline and Rifampicin, according to the results of antibiotic susceptibility tests. Vancomycin, Tigecycline and Trimethoprim/Sulfamethoxazole were the most effective antibiotics against the majority of isolates. Treating antibiotic-resistant bacteria is a clinical challenge, and examining the susceptibility pattern is helpful to identify the future challenges of effective therapy. Staphylococcus spp. have developed resistance to both modern and conventional antibiotics in recent years (Al-Jumaily *et al*.,

2019). The results of the current study showed as shown in Table (6) below that all Staphylococcus aureus isolates were 50% resistant to Benzylpenicillin, Clindamycin, Ciprofloxacin, Rifampicin and Tetracycline. whereas 100% maximum sensitive to Gentamicin, Teicoplanin, Oxacillin, Trimethoprim/Sulfamethoxazole Tigecycline and Vancomycin. The results of this study somewhat agreed with the findings of another study by (Kareem *et al*.,2015) in Diyala, which found gentamycin sensitive 76%, whereas, disagreed with other results of previous study by (Kareem *et al*.,2015, Jia *et a*l.,2020) showed high resistance to Tetracycline and Oxacillin 100%. Previous study by(Akbar *et al*., 2017) showed relative activity of Erythromycins against the tested *S. aureus* isolates were sensitive agreed with our study. Another previous study by (Toner *et al*.,2017) in Pakistan, (He *et al*., 2018) in Australia and (Ahmed *et al*., 2021) in China found that none of *Staphylococcus aureus* isolates resistance for Vancomycin and Tigecycline antibiotic which also agreed with our study. The study also shows that the MICs of *Staphylococcus. aureus* isolates were listed in table (6) below.

A few years prior, it was discovered that the effectiveness of many antibiotics in treating Staphylococcus spp. infections was decreasing, resulting in treatment failure. Staphylococcus species exhibits multiple resistance mechanisms, including the expression of β-lactamases and efflux pumps, which provide a notable level of resistance to clinically relevant antibiotics (Oliveira-Tintino *et al*., 2023). The high minimum inhibitory concentration (MIC) is caused by both the high production of the enzyme extended spectrum β-lactamase (ESBLs), which hydrolyzes the β-lactam ring, and the presence of resistance genes, which prevent the antibiotic from working (Asante *et al*., 2022).

On the other hand, the results of AST-tests and MICs profiles regarding Coagulase-Negative Staphylococcus(**CoNS**) revealed most bacterial isolates were highly resistant to antibiotics, especially Benzylpenicillin, Clindamycin, Gentamicin, Erythromycin, Ciprofloxacin, Oxacillin, Teicoplanin, Tetracycline, Rifampicin, Oxacillin and Tetracycline, whereas, 100% sensitive to Tigecycline and Vancomycin. Also CoNS isolates were 75% high sensitive to Trimethoprim/Sulfamethoxazole.

According to (França *et al*., 2023), **CoNS** strains are most frequently linked to high vaginal bacterial infection and are the most frequently isolated from infections associated with healthcare settings, particularly catheter-related bacteremia and cardiovascular infections. Because of the ability of these isolates to form biofilms can facilitate their persistence in the local site or even in the systemic circulation upon dissemination lead to emergence of multiple bacteria resistant to antibiotics like Gentamicin, Teicoplanin, Benzylpenicillin and Tetracycline(França *et al*., 2023).

In addition to providing protection against antibiotics, the production of biofilm cells plays a significant role in defence against host immune system attacks by reducing the ability to activate the innate immune system and thereby reducing the inflammatory response (Ali *et al*., 2023).

The ability of **CoNS** isolates to secrete exoenzymes like metalloprotease and produce toxins like phenol-soluble modulins (PSMs) is crucial for defence against the host immune system response, especially against neutrophils and antimicrobial peptides (AMPs). Additionally, S. epidermidis generates lipases that aid in the survival of bacteria in fatty acid secretions from the host .

All of which are risk factors for the bacteria's ability to survive longer inside the host body, the immune system's resistance, and the resulting development of antibiotic-resistant bacterial strains. The study also shows

that the MICs of CoNS isolates were listed in Table(6) below.

Regarding ***E. fecalis***, the results of the study as shown in Table (6) below shows the results of an antimicrobial sensitivity test were 100% to Benzylpenicillin, Ciprofloxacin, Teicoplanin and Tigecycline whereas, it was 100% resistant to Clindamycin, Erythromycin, Ciprofloxacin, Oxacillin, Rifampicin, Tetracycline and Trimethoprim/Sulfamethoxazole.

Enterococcus spp. belong to the intestinal microbiota in humans and are found on skin and in smaller proportions in oropharyngeal and vaginal secretions. Urinary tract infections, high vaginal infections, endocarditis, and bacteremia are among the many clinical infections that can be brought on by *E. faecalis* and *E. faecium* (Daca *et a*l., 2024) And, due to the current status of limited antibiotic discovery besides the inappropriate use (misuse and overuse) of available antibiotics are major contributors to the rise of antimicrobial resistance [58].

AST And MICs Profile For Gram-Negative Bacteria

Most Gram-negative bacterial isolates were resistant to most antibiotics, including Cefepime, Ceftazidime, Ciprofloxacin, Gentamicin, Piperacillin, Piperacillin/Tazobactam, Ticarcillin and Ticarcillin/Clavulanic Acid, according to the results of antibiotic susceptibility tests. Amikacin, Imipenem, Meropenem and Trimethoprim/Sulfamethoxazole were the most effective antibiotics against the majority of isolates. According to the the results of AST-tests and MICs as shown in Table(7) below, revealed *E. coli* isolates were 100% resistant to Ceftazidime, Piperacillin, Piperacillin/Tazobactam, Ticarcillin and Ticarcillin/Clavulanic Acid whereas, 100% maximum sensitive towards Amikacin, Imipenem and Meropenem. As for *K.pneumonia* isolates were 100%

resistant to Cefepime, Ceftazidime,Ciprofloxacin, Piperacillin, Piperacillin/Tazobactam, Ticarcillin, Ticarcillin/Clavulanic Acid, Trimethoprim/Sulfamethoxazole and Tobramycin. All of these isolates mentioned above were 100% sensitive to towards Amikacin, Gentamicin, Imipenem and Meropenem. The results of a previous study carried out in the Kurdistan Region of Iraq confirmed the previous findings, indicating that all isolates of *K. pneumonia* were 100% resistant to cefepime, cefotaxime, and ceftazidime. Another study by(Seman *et al.*, 2022) reported different percentages of sensitive towards Amikacin, Ciprofloxacin, Piperacillin/tazobactam, Gentamicin and Trimethoprime-sulfamethoxazole, these results agreed with the findings of this study. In Ethiopia(Zhang *et al*., 2019)found that antibiotics Meropenem, Imipenem, Ceftazidime/Avibactam, and Ceftolozane/Tazobactam had higher sensitivity against Carbape nemase-producing and Extended Spectrum β-Lactamase Enterobacteriaceae isolates.

According to the results of AST-tests and MICs profile as shown in Table(8) below, revealed *P. stutzeri* isolates were 100% resistant to Ciprofloxacin, Piperacillin, Piperacillin/Tazobactam, Ticarcillin, Trimethoprim/Sulfamethoxazole and Tobramycin whereas, 100% maximum sensitive towards Amikacin, Cefepime, Ceftazidime, Gentamicin, Imipenem, Meropenem and Ticarcillin/Clavulanic Acid.

As for *Proteus. mirabilis* isolates were 100% resistant Ciprofloxacin, Imipenem, Piperacillin, Ticarcillin, Trimethoprim/ Sulfamethoxazole, Tobramycin and Ticarcillin/Clavulanic Acid whereas, 100% maximum sensitive towards Amikacin, Cefepime, Ceftazidime, Meropenem and Piperacillin/Tazobactam.

Researchers in the City of Mosul, Iraq (Younus *et al.*, 2022) observed and reported the prevalence of the multidrug-resistant phenomenon of both Gram-positive and Gram-negative bacteria. The high prevalence of multidrug-resistant bacteria found in our study could be attributed to a variety of factors, including microbial traits, selective pressure on antimicrobial use, irrational antibiotic intake, the spread of resistant isolates among individuals, self-medication and medication non-compliance, and sales of substandard antibiotics. One of the major problems in modern medicine is the increasing resistance of pathogenic bacteria to antibiotics. Clinical usage of the currently available antibiotics has suffered from a number of problems, like the development of resistance to one or several antibiotics caused by pathogens capability of modifications and mutations that minimize or remove the contacts between the antibiotics and their target (Baylay *et al*., 2019).

A previous study by (Ahmed *et al*.,2021) showed MDR bacteria have also been shown to cause significant clinical issues in outpatient departments; infections caused by MDR bacteria are associated with a higher mortality rate than infections caused by susceptible bacteria, and they impose significant economic costs. The increase in MDR bacteria worldwide is concerning because it indicates that we are gradually losing our therapeutic options for treating simple bacterial infections.

Beta-lactam antibiotics disrupt the bacterial cell wall synthesis process, specifically targeting peptidoglycan, which is the main component of the cell wall. Consequently, the bacterial resistance to this class of antibiotics arises from the production of Extended-spectrum β-lactamase (ESBL), which breaks down the B-lactam antibiotic ring. Additionally, resistance may arise from a lack of affinity between the antibiotics and their target, such as penicillin-binding proteins (PBP)(Kakoullis *et al*.,2021).

The aminoglycoside group of antibiotics works by binding to the small unit 30s of the bacterial ribosome, which blocks the bacteria's ability to synthesize proteins. This ultimately results in the death of the bacteria. The resistance of this group of antibiotics is caused by the bacteria's possession of efflux systems, antibiotic-modifying enzymes, and other mechanisms of resistance, such as target site modification and mutations. This group of antibiotics targets DNA gyrase and topoisomerase IV, but the resistance of bacteria to this group of antibiotics is caused by the emergence of a mutation in the target site, which is represented by the replication enzymes(Kong *et al*., 2020).

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