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RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF BACTERIA STRAINS IN CATHETER-RELATED BLOODSTREAM INFECTION AMONG THE HEMODIALYSIS EGYPTIAN PATIENTS

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Abstract

Bacterial infections are a significant threat to individuals with kidney diseases, especially those undergoing hemodialysis. Catheter-related bloodstream infection (CRBSI) is a common and serious concern in these patients. Inserted catheters quickly become coated with plasma proteins, providing an opportunity for bacteria to migrate from the skin through the catheter track or from the catheter hubs into this protein sheath. This study aimed to identify and characterize causative organisms in CRBSI from hemodialysis Egyptian patients, utilizing 16S rRNA sequencing. 4 different bacterial strains were successfully identified including*Staphylococcus* sp., *Bacillus* sp., *Enterococcus* sp., and *Delftia* sp. using microbial identification in addition to 16S rRNA sequencing. Whole genome sequencing of *Delftiatsuruhatensis* and *Enterococcus faecalis* was also performed to gain deeper insights into the genetic characteristics of these bacterial isolates. The study revealed genes associated with antimicrobial resistance in these two bacterial strains. Precise bacterial identification is crucial for guiding patient care and selecting appropriate antibiotic treatments, providing reassurance to clinicians, and aiding in treatment adjustment based on antibiotic sensitivity.Importantly, it should be noted that few studies covered this in Egypt, emphasizing the significance of this study in addressing a critical healthcare concern in the region.

Introduction:-

Chronic kidney disease (CKD) stands as a significant global health concern, with its prevalence steadily rising [1, 2]. This multifaceted condition encompasses a spectrum of stages, from mild kidney dysfunction to end-stage renal disease, demanding tailored management approaches [3]. Classification and understanding of CKD have evolved over the years, leading to improved epidemiological insights and delineation of risk factors [4, 5]. The intricate pathophysiology of CKD involves a network of biological mechanisms and various etiological factors, underlining

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the importance of early diagnosis and intervention [6, 7]. The management of CKD represents a substantial challenge in modern healthcare, and understanding it is pivotal in providing effective patient care, as it encompasses a broad spectrum of disease severity and risk factors [2].

Patients with CKD often progress to end-stage renal disease, necessitating renal replacement therapies (RRT) such as hemodialysis [8, 9]. Hemodialysis, a life-saving renal replacement therapy, plays a pivotal role in the management of end-stage renal disease and requires meticulous attention to detail [1, 9]. This includes comprehending the epidemiological trends related to dialysis, the diverse types of dialysis available, the underlying mechanisms of hemodialysis, and its associated outcomes, notably mortality, cardiovascular disease, vascular access-related complications, and infection [8, 10]. Hemodialysis aims to restore electrolyte balance and remove waste products from the bloodstream [9].

Vascular access plays a pivotal role in hemodialysis, influencing both the effectiveness of the treatment and the patient's overall well-being [9]. Catheters, both for extracorporeal renal replacement therapy and peritoneal dialysis, serve as indispensable tools in the hemodialysis process [11]. These catheters come in various forms, each tailored to specific clinical scenarios. Understanding the different catheter types, insertion techniques, and appropriate care is essential to minimize complications. However, the use of catheters is not without risks [12, 13]. Complications arising from catheter insertion can have short-term and long-term implications [12]. The short-term complications encompass a wide array, including pleural puncture, arterial puncture, bleeding, arrhythmias, air embolism, and central venous injuries. Long-term complications, such as infections, catheter malfunction, fibrin sheath formation, thrombosis, tip malposition, and central venous stenosis, necessitate continuous monitoring and management [12, 14].

Among the potential complications, catheter-related bloodstream infections (CRBSIs) are of principal concern. These infections pose a significant threat to patients undergoing dialysis, requiring careful diagnosis and management [13, 15]. The diagnosis of CRBSI is a multifaceted process, involving clinical judgment, microbiological assessments, and, in some cases, catheter withdrawal. The emergence of antimicrobial resistance further complicates the management of CRBSI, necessitating a nuanced approach to antibiotic therapy [16, 17].

By addressing these multifaceted issues, we aim in this study to investigate and identify bacterial strains isolated from blood catheters of hemodialysis patients to assess improving patient outcomes and the quality of care provided to individuals facing kidney-related health challenges.

Methods:-

Patients and Samples

Approval for the study was obtained from the Research Ethical Committee, Faculty of Pharmacy, Suez Canal University (202301_MH1), and written informed consent was obtained from all participants. This study focused on adult hemodialysis patients treated at various governmental hospitals in Cairo, Egypt. These patients had recently undergone the removal of temporary central venous catheters (CVC) using sterile techniques. Inclusion criteria for hemodialysis patients were taken into consideration which indicates the possibility of infection. Data collected included patient names, gender, age, hospital, and the duration of inclusion criteria signs before sample collection.

Bacterial Isolates Identification and Characterization

Nutrient Agar (NA) (g/l) Beef extract 3.0, Peptone 5.0, Sodium chloride 5.0, and Agar 15.0) was used as the primary medium for cultivating bacterial strains from patient samples. Once pure bacterial cultures were obtained, the morphological identification using the Gram stain technique was performed. Selective and differential media were used to identify specific traits and metabolic activities of the isolates, including Blood Agar (BA) (g/l)(Casein enzymic hydrolysate 14.0, Peptic digest of animal tissue 4.5, Yeast extract 4.5, Sodium chloride 5.0, Agar 12.5 and final pH (at 25°C) 7.3), Mannitol Salt Agar (MSA) (Beef extract 1.0, Peptone 10.0, NaCl 75.0, Mannitol 10.0, Phenol red 0.025, Agar 15.0), MacConkey Agar (MAC) (Peptone 17.0, Proteose peptone 3.0, Lactose 10.0, Bile salts 1.5, Sodium chloride 5.0, Neutral red 0.03, Crystal violet 0.001, Agar 13.5), and Cetrimide Agar (CA) (Potassium chloride 10.0, Cetrimide (cetyltrimethylammonium bromide) 0.3, Glycerol 10 mL, Agar13.6).

Molecular Identification of Bacterial Isolates

Identification of bacterial isolate using 16S rRNA sequencing

DNA extraction was performed according to the manufacturer's protocol using a GeneDireX genomic DNA isolation kit (GeneDirex, Taiwan Cat. No. NA023-0100). The concentration and purity of the extracted DNA were assessed using a Nanodrop C2000 system (ThermoFisher Scientific, USA).

The Primers 5'-AGAGTTT-GATCMTGGCTCAG-3' and 5'-TACGGYTACCTTGTTACGACTT-3' were used to amplify nearly full-length 16S rRNA gene. The design of the primers was performed by the Integrated DNA Technology (IDT) primer quest tool (https://eu.idtdna.com/pages/tools/primerques). PCR was carried out using DreamTaq Green PCR MasterMix (2X) (ThermoFisher Scientific). Genomic DNA was diluted to 30 ng/µl, and primers were prepared at 10 pmol concentrations. The reaction mixture was prepared according to the manufacturer's protocol and the reactions were conducted in a T100 Thermal Cycler (Bio-Rad, USA). PCR products were analyzed through Agarose Gel Electrophoresis (1%). Electrophoresis was performed, and the gel was visualized using a gel documentation system for quality assessment and DNA concentration estimation. The purification of 16S rRNA PCR products was performed using the GeneJet PCR Purification Kit (ThermoFisher Scientific, USA, Cat. No. K0701) for Sanger sequencing.

Sequencing of 16S rRNA PCR Products Using Sanger Technology

A BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems, ThermoFisher Scientific, USA, Cat. No. 4337454) was used to sequence the amplification products. The cycle sequencing process (95°C for 1 min, followed by 25 cycles of 95°C for 10 s, 59°C for 5 s, and 72°C for 4 min) was carried out using the GeneAmp™ PCR System 9700 Thermal Cycler (ThermoFisher Scientific, USA, Cat. No. 4339386). Sequencing products were purified by the BigDyeXTerminator™ Purification Kit (ThermoFisher Scientific, USA, Cat. No. 4376484) after the cycle sequencing step. The 3500XL Genetic Analyzer from Applied Biosystems (ThermoFisher Scientific, USA) was utilized for capillary electrophoresis, and the data collected during this process were prepared for subsequent analysis.The raw files containing sequence data were collected, and their signal intensities were checked to ensure data quality. The data were then visualized using FinshTV 1.4.0 and SnapGene, while Sequencher 4.0 was employed to trim the sequences' ends. High signal intensities were indicative of high-quality data, signifying that the sequencing reactions produced a sufficient amount of labeled product for accurate base calling. A portion of the initial sequence (approximately 70-80 bases) was identified as noisy and subsequently trimmed using analysis software. Subsequently, NCBI/BLAST (http://www.ncbi.nlm.nih.gov/BLAST) was utilized to explore sequence similarities and generate phylogenetic trees for visualization. Finally, the sequence data files were submitted to NCBI.

Antimicrobial Resistance Characterization Using Disc Diffusion Method

The antimicrobial susceptibility of isolates was assessed following the Clinical and Laboratory Standards Institute (CLSI) standard approach[18], utilizing the disk diffusion method and Mueller-Hinton (MH) agar as the culture medium. These measurements were used to classify the bacterial isolates as either sensitive (S), intermediate (I), or resistant (R) to the antibiotics, in accordance with the guidelines provided by the CLSI, as outlined in tables (4-7). To prepare the bacterial isolates for antimicrobial susceptibility testing (AST), they were initially cultured on Nutrient agar and incubated at 37°C for a period of 24 to 48 hours. Subsequently, colonies were selected and suspended in 5 ml of saline solution. 15 antibiotics were chosen as shown in **table (1)** (OxiodThermoFisher Scientific, USA), and bacterial colonies from nutrient agar were suspended in saline to a specific turbidity. This suspension was streaked on MH agar (g/l) (Beef Extract 2.0, Acid Hydrolysate of Casein 17.5, Starch 1.50, Agar 17.0), and antibiotic disks were added. The plates were then incubated at 37°C. After the incubation period, the zones of inhibition around each antibiotic disc were carefully measured in millimeters (mm).

Table 1:- Details of antibiotics used.

Abbreviation	Antibiotic	Classification	Disc load	Cat. No.
MEM-10	Meropenem	Carbapenems Beta-lactams	30 µg	R21156
CAZ-30	Ceftazidime	Third-generation cephalosporin class of beta-lactams	2 µg	CT0064B
CTX-30	Cefotaxime		15 µg	CT0020B
IPM-10	Imipenem	Beta-lactams	10 µg	CT0774B
TPZ-110	Piperacillin/Tazobactam		30 µg	CT0412B
P-10	Penicillin		30 µg	CT0166B

AM-10	Ampicillin	Penicillin group of β-lactam antibiotics	10 µg	CT0455B
VA-30	Vancomycin	Glycopeptide	10 µg	R21136
TEC-30	Teicoplanin		30 µg	CT0647B
S-10	Streptomycin	Aminoglycoside antibiotics	10 µg	CT0047B
CLR-15	Clarithromycin	Macrolide group	15 µg	CT0693B
RA-5	Rifampin	Antimycobacterial	5 µg	R21139
L-2	Lincomycin	Lincosamide class	2 µg	CT0028B
DA-2	Clindamycin		10 µg	CT0003B
E-15	Erythromycin	Macrolide antibiotics	10 µg	CT0725B

Bacterial Isolates Characterization Using Vitek2 System

Before proceeding to whole genome sequencing, selected bacterial isolates were subjected to confirmatory identification using the VITEK 2 Compact system (bioMérieux, France), which is an automated system for studying microorganisms that uses growth-based technologies. Also, it depends on identical colorimetric reagent cards, which are automatically incubated and read. Gram-negative fermenting and non-fermenting bacilli (GN) and Gram-positive cocci and non-spore-forming bacilli (GP) Vitek 2 compact cards were used.

Whole genome sequencing of bacterial isolates

Library Amplification and Genome Sequencing

Genomic DNA extraction was conducted using the GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific, USA). The quality of DNA extraction was assessed using the Nanodrop C2000 system (ThermoFisher Scientific, USA). The sequencing library was prepared by random DNA fragmentation, followed by the ligation of 5' and 3' adapters using the truseq nano DNA library kit (Illumina, USA). The size of PCR-enriched fragments was verified through electrophoresis on an Agilent Technologies 2100 Bioanalyzer (Agilent, USA).For cluster generation, the prepared library was loaded onto a flow cell, where fragments were captured by surface-bound oligos that are complementary to the library adapters. Each fragment was then amplified into distinct, clonal clusters via bridge amplification.Subsequently, sequencing was conducted using the Illumina Novaseq6000 device (Illumina, USA).

Genome Assembly and Genome Annotation

FastQC (V0.11.8) was employed to verify the quality of the high-throughput sequencing data. After that, Trimmomatic[19] was utilized to filter out adapter sequences and eliminate low-quality reads from the raw fastaq files. The filtered reads were then mapped to the reference genome of *Delftiatsuruhatensis* and *Enterococcusfaecalis*using BWA-MEM (Galaxy Version 0.7.17.1) [20]. SAMTools was then applied to manipulate the SAM/BAM files generated by the BWA-MEM software [21]. The identification of variants, including single nucleotide polymorphisms (SNPs) and short insertions or deletions (indels), was accomplished through SnpEff (v4.3t) [22]. Finally, genome annotation was conducted using two different tools: Prokka, a software for prokaryotic genome annotation [23], and RAST (Rapid Annotation using Subsystem Technology) [24].

Phylogenetic Analysis

Sequencher, a software provided by Gene Code Corporation (MI, USA), was utilized to edit and assemble the 16S rRNA sequencing reads. To obtain the relevant sequences, the nucleotide BLAST program available at https://blast.ncbi.nlm.nih.gov/Blast.cgi was employed, and these sequences were then aligned using ClustalW version 1.8 [25]. For the identification of housekeeping genes, a BLAST search in GenBank (www.ncbi.nlm.nih.gov) using the nucleotide BLAST program was performed, followed by alignment using ClustalW version 1.8 [25]. The phylogenetic analyses were conducted using the maximum likelihood (ML) algorithm [26] and implemented in MEGA 6.06 [27]. The Tamura-Nei model was used for these analyses. To assess the support for each node, bootstrap support (BT) was calculated through 1000 replicates.

Results:-

A total of n=38 samples were collected from temporary central venous catheters (CVC) of hemodialysis patients during the study with suspected CRBSI among adult patients age ranging from 18 to 84 years with a mean and median age of 46 years and 49 years, respectively. The distribution of male and female patients was 44.74% and 55.26%, respectively. Out of a total of 38 blood samples from CVC catheters, 15 blood samples showed positive bacterial growth which represented 47.37% of the total collected samples. Among these positive cultures, 44.44%

were male patients and 55.55% were female patients. The total number of patients with detailed records is shown in **Table (2)**.

Table 2:- Samples' record; code, gender, age, location of collection.

Isolate ID	Gender	Age	Location of collection	Bacterial strains
Gram-positive bacteria				
Mat-1	Male	47	Cairo	*Enterococcus* sp.
Mat-2	Male	84	Cairo	*Enterococcus* sp.
Oct-3	Male	63	Giza	*Bacillus* sp.
Mat-4	Female	64	Cairo	Coagulase-negative *Staphylococcus*
Mat-5	Female	57	Cairo	*Bacillus* sp.
Oct-6	Female	52	Giza	Coagulase-positive *Staphylococcus*
Mat-7	Male	43	Cairo	*Enterococcus* sp.
Mat-8	Female	41	Cairo	*Enterococcus* sp.
Mat-9	Female	55	Cairo	Coagulase-positive *Staphylococcus*
Mat-10	Female	39	Cairo	Coagulase-positive *Staphylococcus*
Mat-13	Male	54	Cairo	*Enterococcus* sp.
Mat-14	Female	62	Cairo	Coagulase-positive *Staphylococcus*
Oct-15	Female	51	Giza	Coagulase-negative *Staphylococcus*
Gram-negative bacteria				
Mat-11	Male	20	Cairo	*Delftia* sp.
Mat-12	Female	44	Cairo	*Delftia* sp.

Following gram staining and microscopic analysis of isolated samples, the results revealed that 13 of these samples exhibited a Gram-positive staining pattern, whereas the remaining 2 samples exhibited a Gram-negative staining pattern (as shown in **Table 2**). The Gram-positive bacteria identified were *Staphylococcus*spp, *Enterococcus*spp, and *Bacillus*spp, while the Gram-negative category included *Delftia* spp. This was according to the microscopic examination, where it was observed that the Gram-negative strains displayed motility characteristics, while the Gram-positive strains were characterized as immotile bacteria.

Following microbial characterization, 16S rRNA sequencing was performed. Sequence analysis and alignment were carried out by comparing trimmed fragment sequences to the NCBI database using NCBI/BLAST/blastn. The alignment resultsshowed high percentages of query coverage and percent identity, along with zero E-values, indicating substantial sequence similarity to entries in the NCBI database as shown in **Table (3)**.In this study, seven distinct bacterial strains were isolated from the study samples, including *Enterococcusfaecalis* (found in five samples), *Staphylococcusaureus* (found in four samples), *Bacilluscereus* (found in two samples), *Staphylococcushaemolyticus* (found in two samples) and *Delftiatsuruhatensis* (found in two samples) (**Table 3, Figure 1**). After successful sequence alignment and isolate identification, the sequence fragments were submitted to the NCBI databases for reference (**Table 3**).

Table 3:-Represents bacterial isolates identification and its NCBI accession number.

Isolates ID	16S rDNA				
	Sequence (bp)	Accession no.	Homology to the reference strains		Identity (%)
Mat-1	967	OQ826448	*E. faecalis*TMPC	ON778623.1	99.18
Mat-2	964	OQ826449	*E. faecalis*TMPC	ON782136.1	99.59
Oct-3	984	OQ826450	*B. cereus*ZR452	OR294189.1	99.79
Mat-4	1391	OQ826452	*S. haemolyticus*PL565	MT539735.1	99.86
Mat-5	1277	OQ826453	*B. cereus*AZ-3	KT270479.1	90.06
Oct-6	1318	OQ826455	*S. aureus*UNC_SA55	CP132362.1	99.98
Mat-7	983	OQ826456	*E. faecalis* V1876	OR432331.1	99.80
Mat-8	1389	OQ826457	*E. faecalis* PM11	OP431821.1	99.78
Mat-9	867	OQ826458	*S. aureus* NL1	CP077741.1	99.54
Mat-10	959	OQ826460	*S. aureus* 14507	CP053356.1	99.17

Mat-11	920	OQ826461	*D. tsuruhatensis*TR39T1	KJ206921.1	100
Mat-12	912	OQ826462	*D. tsuruhatensis*TR39T1	KJ206921.1	99.78
Mat-13	1271	OQ826463	*E. faecalis*Jilu LG128	KX904725.1	99.29
Mat-14	1456	OQ826464	*S. aureus* LKY-Strp01	MK886615.1	99.86
Oct-15	1388	OQ826465	*S. haemolyticus*PL565	MT539735.1	99.71

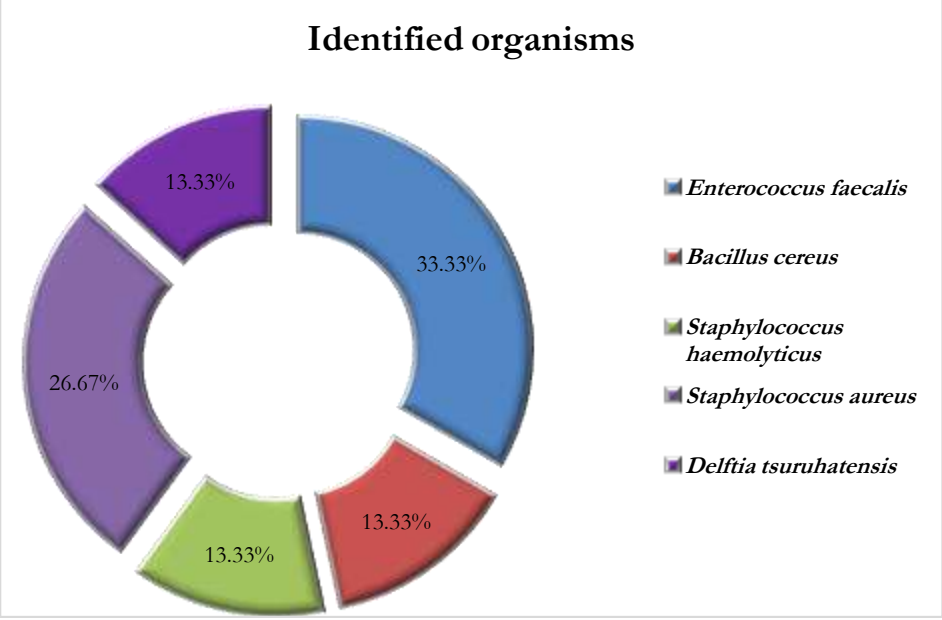


Figure 1:- Represents the bacterial isolates. Five different bacterial strains had been identified as follows: *Enterococcusfaecalis* represented 33.33% of bacterial isolates from all experimental sample sizes. While *Staphylococcusaureus* represented 26.67% of bacterial isolates. Moreover, each of *Bacilluscereus, Staphylococcushemolytics*,and*Delftiatsuruhatensis* represent 13.33% of the total isolates.

The bacterial isolates obtained from patients' samples were thoroughly characterized and subjected to antimicrobial sensitivity and susceptibility tests. These tests were conducted to assess how these bacterial isolates respond to various specific antibiotics, allowing us to determine the presence of antimicrobial resistance among the bacterial isolates within our sample size (**Tables (4-8)** and **Figure 4**).

Table 4:- Illustrates *Staphylococcusaureus* bacterial isolates' response to antibiotics.Inhibition zone in mm. Antibiotic response interpretation is expressed in S for sensitivity and R for resistance.

Staphylococcus aureus								
Antibiotics	Oct-6		Mat-9		Mat-10		Mat-14	
Vancomycin	23	S	17	S	17	S	24	S
Clindamycin	35	S	30	S	28	S	37	S
Erythromycin	30	S	30	S	20	S	20	S
Meropenem	26	S	0	R	0	R	12	R
Ceftazidime	0	R	0	R	0	R	0	R
Cefotaxime	10	R	13	R	14	R	0	R
Imipenem	35	S	20	S	40	S	0	R
Penicillin	15	S	3	R	12	R	0	R
Teicoplanin	18	S	20	S	16	S	25	S
Streptomycin	15	S	14	R	15	S	25	S
Clarithromycin	31	S	30	S	25	S	30	S
Rifampin	30	S	20	S	25	S	18	S
Lincomycin	30	S	25	S	22	S	36	S
Ampicillin	0	R	0	R	12	R	0	R

Piperacillin/Tazobactam	20	S	15	S	20	S	0	R

Table 5:- Illustrates *Enterococcusfaecalis* bacterial isolates' response to antibiotics. Inhibition zone in mm. Antibiotic response interpretation is expressed in S for sensitivity and R for resistance.

Enterococcus faecalis									
Antibiotics	Mat-1		Mat-2		Mat-7		Mat-8		Mat-13	
Vancomycin	30	S	36	S	18	R	18	R	20	S
Clindamycin	22	S	31	S	27	S	0	R	0	R
Erythromycin	12	R	40	S	6	R	27	S	30	S
Meropenem	28	S	37	S	35	S	18	R	22	S
Ceftazidime	0	R	30	S	0	R	0	R	0	R
Cefotaxime	20	S	27	S	25	S	20	S	0	R
Imipenem	30	S	24	S	12	R	32	S	25	S
Penicillin	30	S	40	S	19	S	16	R	25	S
Teicoplanin	20	S	31	S	19	S	16	S	20	S
Streptomycin	27	S	26	S	13	R	0	R	0	R
Clarithromycin	0	R	29	S	10	S	20	S	30	S
Rifampin	34	S	35	S	30	S	11	R	20	S
Lincomycin	34	S	24	S	16	S	0	R	0	R
Ampicillin	10	R	28	S	14	R	22	R	0	R
Piperacillin/Tazobactam	24	S	25	S	13	R	30	S	25	S

Table 6:- Illustrates *Staphylococcushemolytics* bacterial isolates' response to antibiotics.Inhibition zone in mm. Antibiotic response interpretation is expressed in S for sensitivity and R for resistance.

Staphylococcus hemolytics				
Antibiotics	Mat-4		Oct-15	
Vancomycin	24	S	0	R
Clindamycin	30	S	0	R
Erythromycin	21	S	11	R
Meropenem	19	S	30	S
Ceftazidime	0	R	0	R
Cefotaxime	0	R	0	R
Imipenem	37	S	35	S
Penicillin	10	R	20	S
Teicoplanin	20	S	20	S
Streptomycin	20	S	20	S
Clarithromycin	35	S	36	R
Rifampin	15	R	30	S
Lincomycin	24	R	31	S
Ampicillin	0	R	0	R
Piperacillin/Tazobactam	0	R	20	S

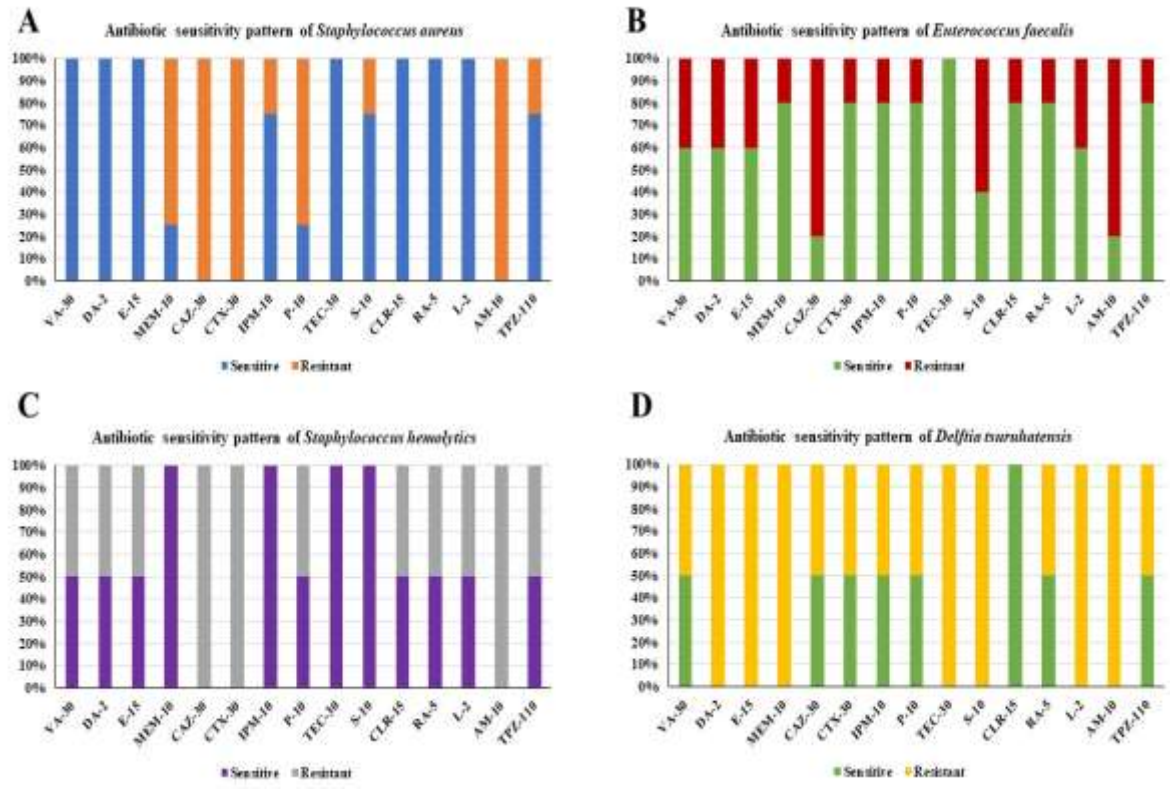
Table 7:- Illustrates *Bacilluscereus* bacterial isolates' response to antibiotics. Inhibition zone in mm. Antibiotic response interpretation is expressed in S for sensitivity and R for resistance.

Bacilluscereus				
Antibiotics	Oct-3		Mat-5	
Vancomycin	25	S	40	S
Clindamycin	20	S	38	S
Erythromycin	30	S	32	S
Meropenem	10	R	34	S
Ceftazidime	0	R	32	S
Cefotaxime	25	S	37	S
Imipenem	20	S	29	S
Penicillin	40	S	40	S

Teicoplanin	16	S	40	S
Streptomycin	20	S	40	S
Clarithromycin	20	S	37	S
Rifampin	20	S	30	S
Lincomycin	20	S	40	S
Ampicillin	10	R	32	S
Piperacillin/Tazobactam	21	S	40	S

Table 8:- Illustrates *Delftiatsuruhatensis* bacterial isolates' response to antibiotics.Inhibition zone in mm. Antibiotic response interpretation is expressed in S for sensitivity and R for resistance.

Delftiatsuruhatensis				
Antibiotics	Mat-11		Mat-12	
Vancomycin	16	S	0	R
Clindamycin	6	R	0	R
Erythromycin	14	R	12	R
Meropenem	0	R	0	R
Ceftazidime	20	S	0	R
Cefotaxime	30	S	14	R
Imipenem	16	R	34	S
Penicillin	25	S	0	R
Teicoplanin	6	R	0	R
Streptomycin	15	R	0	R
Clarithromycin	25	S	20	S
Rifampin	23	S	10	R
Lincomycin	0	R	0	R
Ampicillin	15	R	10	R
Piperacillin/Tazobactam	25	S	0	R



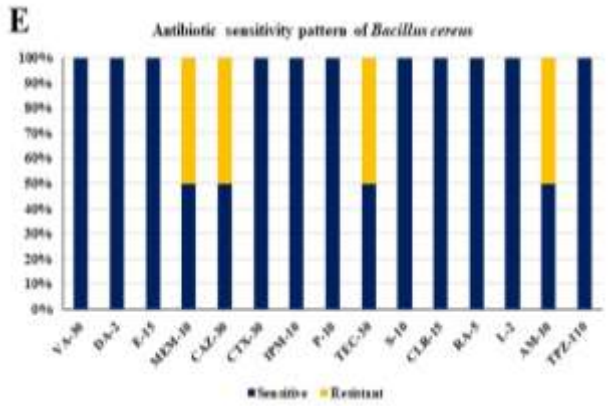


Figure 2:- Represents antibiotic sensitivity patterns of bacterial isolates. (A) antibiotic sensitivity patterns of *Staphylococcus aureus*. (B) antibiotic sensitivity patterns of *Enterococcusfaecalis*. (C) antibiotic sensitivity patterns of *Staphylococcushemolytics*. (D) antibiotic sensitivity patterns of *Delftiatsuruhatensis*. (E) antibiotic sensitivity patterns of *Bacilluscereus*.

Table 9 represents the antibiograms of gram-positive and gram-negative bacterial sensitivity testing. Each column represents the species of bacteria tested and the total number of bacteria isolated. The rows represent the different types of antibiotics tested for. Each bacteria-antibiotic combination was represented by the percentage of organisms sensitive to its antibiotic. Only Teicoplanin was fully efficacious against all Gram-positive bacteria from the antibiogram. Clarithromycin was the most effective antibiotic with 100% sensitivity against gram-negative bacteria tested.

Table 9:- Antibiogram for bacterial isolates.

Bacteria	***Staphylococcus aureus***	***Enterococcus faecalis***	***Staphylococcus hemolytics***	***Bacillus cereus***	***Delftiatsuruhatensis***
No. of isolates	4	5	2	2	2
Percentages (%)					
Vancomycin	100	60	50	100	50
Clindamycin	100	60	50	100	0
Erythromycin	100	60	50	100	0
Meropenem	25	80	100	50	0
Ceftazidime	0	20	0	100	50
Cefotaxime	0	80	0	100	50
Imipenem	75	80	100	100	50
Penicillin	25	80	50	100	50
Teicoplanin	100	100	100	100	0
Streptomycin	75	40	100	100	0
Clarithromycin	100	80	50	100	100
Rifampin	100	80	50	100	50
Lincomycin	100	60	50	100	0
Ampicillin	0	20	0	50	0
Piperacillin/Tazobactam	75	20	50	100	50

Two bacterial strains were selected for Whole-genome Sequencing which were Delftiatsuruhatensis, and Enterococcus faecalis, because of their uncommon presence in catheter-related bloodstream infections in hemodialysis patients. DNA from the two samples was checked for quality control and processed to WGS. Reads for whole genome sequenced bacterial isolates were submitted to the comprehensive genome analysis service at PATRIC. 23,726,204 and 25,585,458 read with 12,321,111 and 2,333,789 bases were generated for Delftiatsuruhatensis and Enterococcus faecalis, respectively. **Table 10** summarizes the resulting sequencing data

analysis, including genome assembly, protein features, and specialty gene annotation. **Table 11** represents the antimicrobial resistance genes and key factors involved in antibiotic resistance mechanisms found in sequenced bacterial isolates. Moreover, the functional categorization (subsystems), and phylogenetic analysis were provided in **Figures (3-5)**.

Table 10:- Represents the genome assembly of sequenced genomes.

Genome assembly		
Assembly details	***Delftiatsuruhatensis***	***Enterococcus faecalis***
Contigs	139	1258
Genome length	12,321,111 bp	2,333,789 bp
GC content	52.16	63.59
Contig N50	329,604	1,859
Protein features		
Hypothetical proteins	3513	2688
Proteins with functional assignments	8917	802
Proteins with EC number assignments	2654	310
Proteins with GO assignments	2235	292
Proteins with Pathway assignments	1940	258
Specialty gene annotation		
Antibiotic Resistance	150	15
Drug Target	26	3
Transporter	53	10
Virulence Factor	64	8

Table 11:- Represents antimicrobial resistance genes found in sequenced bacterial isolates.

AMR Mechanism	**Genes of *Delftiatsuruhatensis***
Antibiotic inactivation enzyme	APH (3')-II/APH (3')-XV, BlaZ family, FosB, Mph(C) family
Antibiotic target in susceptible species	Alr, Ddl, dxr, EF-G, EF-Tu, folA, Dfr, folP, gyrA, gyrB, inhA, fabI, Iso-tRNA, kasA, MurA, rho, rpoB, rpoC, S10p, S12p
Antibiotic target modifying enzyme	RlmA(II)
Antibiotic target protection protein	BcrC, Lsa(A), Sal(A), Tet(M)
Antibiotic target replacement protein	FabK
Efflux pump conferring antibiotic resistance	BceA, BceB, EmrAB-OMF, EmrAB-TolC, MacA, MacB, MdtABC-OMF, MdtABC-TolC, MexCD-OprJ, MexCD-OprJ system, NorA, YkkCD
Gene conferring resistance via absence	gidB
Protein-altering cell wall charge conferring antibiotic resistance	GdpD, MprF, PgsA
Regulator modulating expression of antibiotic resistance genes	BceR, H-NS, LiaF, LiaR, LiaS, OxyR, VanG-type
AMR Mechanism	**Genes of *Enterococcus faecalis***
Antibiotic target in susceptible species	EF-G, EF-Tu, folP, gyrA, gyrB, Iso-tRNA, parE/parY, rpoB, rpoC, S12p
Protein-altering cell wall charge conferring antibiotic resistance	GdpD, PgsA

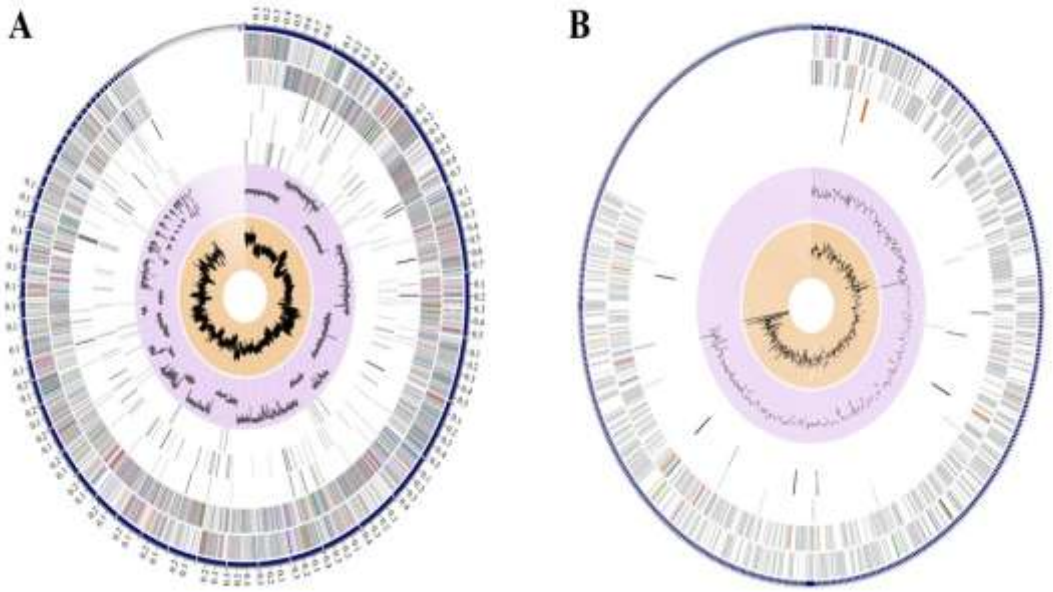


Figure 3:- A circular graphical display of the distribution of the genome annotations. This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to known virulence factors, GC content, and GC skew. (A) *Delftiatsuruhatensis* and (B) *Enterococcusfaecalis*.

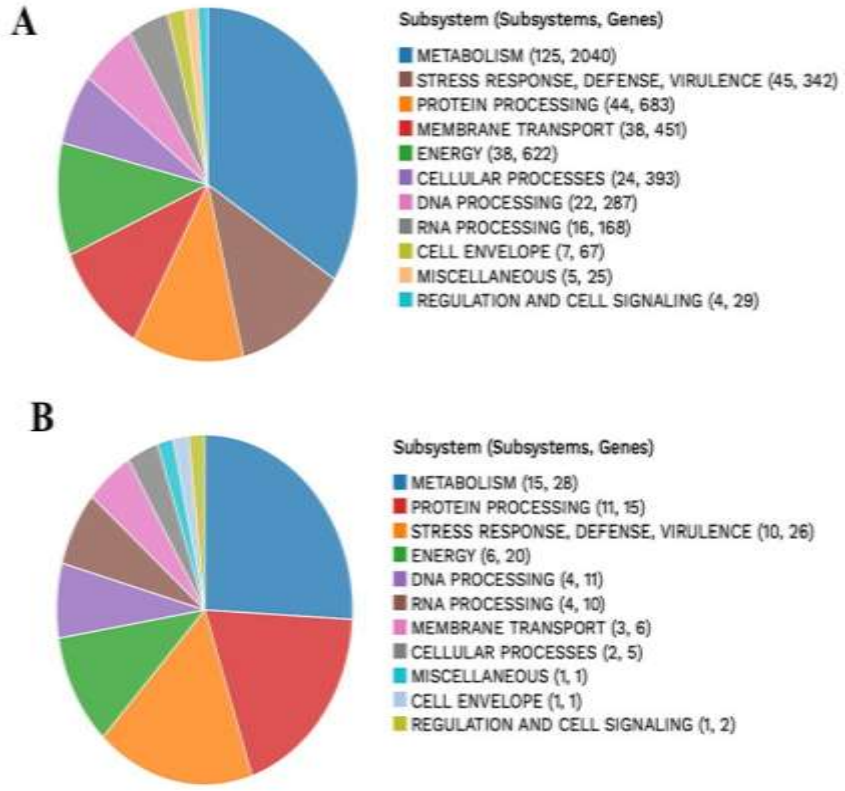


Figure 4:- Represents an overview of subsystem analysis for sequenced genomes of bacterial isolated species. (A) *Delftiatsuruhatensis* and (B) *Enterococcusfaecalis*.

Discussion:-

This study aimed to identify and characterize bacterial strains causing Catheter-Related Bloodstream Infections (CRBSI) in Chronic Kidney patients. Commonly used CRBSI diagnostic methods, like quantitative blood culture and staining are misleading and inaccurate in some cases, while 16S rRNA sequencing and whole-genome sequencing can provide more information about pathogen identification, epidemiology, and drug susceptibility, and are essential for outbreak management [28]. Chronic Kidney patients often require Renal Replacement Therapy (RRT) such as Hemodialysis (HD). HD relies on vascular access through methods like arteriovenous fistulas (AVF), recommended by guidelines. However, in certain cases, Central Venous Catheters (CVC) are used due to practicality [29]. Patients using CVC frequently experience side effects, including infections. CRBSIs, bacterial infections related to catheters, can lead to serious complications, hospitalization, and increased mortality. Consistency in CRBSI definitions is lacking, prompting efforts to standardize these definitions [28, 29].

In this study, the samples werecollected from HD patients with clinical signs of CRBSI, adhering to standardized diagnostic criteria including fever ≥ 38°C (axillary temperature), chills, erythema, purulent discharge from catheter site, tenderness over catheter exit site, clogging of catheter tips (in some cases), elevated C-Reactive Protein (CRP) [28]. Infection risk factors considered clinical care during CVC use, basic conditions, catheter insertion technique, site, time, and goals. Local risk factors like uncleanliness, occlusive dressings, and infections can enhance CRBSI pathogenesis[28]. Notably, few studies covered this clinical topic in Egypt, emphasizing the significance of this study in addressing critical healthcare concerns.

Traditional microbial culturing has limitations, especially in cases with small, slow-growing, or antibiotic-treated bacteria. 16S rRNA gene sequencing has been used for accurate microbial detection. In this study, 16S rRNA sequencing identified five bacterial isolates. The study demonstrated*Enterococcusfaecalis*, *Staphylococcusaureus*,*Bacilluscereus*, and *Staphylococcushaemolyticus*which are common causes of CRBSI[30]. Uncommon bacteria like*Delftiatsuruhatensis* which is rarely identified by traditional methods in CRBSI were also identified in this study. These results underlined the importance of accurate bacterial identification in CRBSI management, where 16S rRNA sequencingoffered rapid, precise identification, overcoming the limitations of traditional methods.

Antimicrobial sensitivity and susceptibility tests were performed on the bacterial isolates from CRBSI in hemodialysis patients. Among the antibiotics used were β-lactams, such as Imipenem, Ampicillin, Penicillin, and Piperacillin/Tazobactam, which inhibit bacterial cell wall formation. Additionally, third-generation cephalosporins like Ceftazidime and Cefotaxime, as well as carbapenems like Meropenem, were used. These antibiotics interfere with bacterial cell wall synthesis and protein production, making them essential for assessing antimicrobial resistance in isolated bacteria. Bacterial strains can develop resistance through various mechanisms, including the production of enzymes like serine-lactamases (for β-lactam resistance), modification of ribosomal targets (for macrolide, lincosamide, and aminoglycoside resistance), and changes in peptidoglycan precursors (for glycopeptide resistance).

Our study revealed that different samples of Staphylococcus aureus responded differently to antibiotics within different classes. Among Staphylococcus aureus isolates, multidrug resistance was observed, with resistance to Ceftazidime, Cefotaxime, and Ampicillin within the β-lactams class. However, some sensitivity to other antibiotic classes like Glycopeptides, Licosamides, Macrolides, and Antimycobacterial antibiotics was noted. *Enterococcusfaecalis*also exhibited multidrug resistance, particularly against third-generation cephalosporin β-lactams (Ceftazidime and Cefotaxime). However, it responded to Meropenem, Imipenem, Penicillin, Piperacillin/Tazobactam, and Teicoplanin. Some resistance to Vancomycin was observed.*Staphylococcushaemolyticus*, isolated from two patients, exhibited resistance to the third-generation cephalosporin β-lactams and Ampicillin but responded to Meropenem and Imipenem. Penicillin and Piperacillin/Tazobactam showed varied responses among these samples. *Bacillus cereus* samples responded to various antibiotics across different classes, including Cefotaxime, Imipenem, Penicillin, Piperacillin/Tazobactam, and other classes, with some resistance observed in certain cases. *Delftiatsuruhatensis*, although relatively uncommon in causing catheter-related bloodstream infections, exhibited multidrug resistance. It resisted Meropenem and Ampicillin but responded to other antibiotics in the study. This underscores the importance of antibiotic susceptibility testing to guide effective treatment strategies.

Vitek 2 system had successfully identified *Enterococcus faecalis*with 99% while*Delftiatsuruhatensis*could not be identified using the same Viteck system. Identification of non-fermentative Gram-negative rods poses a challenge to clinical laboratories since commercial biochemical systems frequently fail to provide an accurate result [31]. Likewise in this case *Delftiatsuruhatensis* was misidentified by the VITEK 2 system as a closely related species, which in routine clinical laboratories can only be distinguished by molecular methods [32]. Hence, if accurate identification is needed, 16S rRNA gene sequencing and whole genome sequencing are recommended.

To explore deeper into the isolated bacterial strains, one sample of *Delftiatsuruhatensis*(Mat-12) was selected for whole genome sequencing due to its uncommon occurrence in CRBSI and its higher multidrugresistance in comparison to the other sample (Mat-11). Additionally, another sample of *Enterococcus faecalis*(Mat-8) was chosen for sequencing due to its high multidrug resistance despite being a well-known cause of CRBSI. Genome annotation was performed using Prokka Prokaryotic genome annotation [23]. The resulting assembly generated 139 contigs, with 12,321,111 bases, with N50 of 329,604 for *Delftiatsuruhatensis*. On the other hand, for *Enterococcusfaecalis*, 1258 contigs with 2,333,789 bases, with N50 of 1859 were reported. The assembly identified 112 and 27 rRNA, 176 and 35 tRNA, and 12,430 and 3490 coding regions for *Delftiatsuruhatensis*and *Enterococcus faecalis*, respectively. The functional annotation was carried out by RAST (Rapid Annotation using Subsystem Technology [24].

The genome analysis of *Delftiatsuruhatensis* revealed the presence of 150 antibiotic resistance genes, 26 drug target genes, 53 transporter genes, and 64 virulence factor genes. In contrast, *Enterococcus faecalis* displayed 15 antibiotic resistance genes, 3 drug target genes, 10 transporter genes, and 8 virulence factor genes. These findings were further supported by an analysis of antimicrobial resistance genes, which indicated the presence of various resistance mechanisms and pathways in these bacterial genomes including antibiotic inactivation enzymes, antibiotic target in susceptible species, antibiotic target modifying enzymes, antibiotic target protection proteins, antibiotic target replacement proteins, efflux pump conferring antibiotic resistance, genes conferring resistance via absence, proteins altering cell wall charge conferring antibiotic resistance, and regulators modulating expression of antibiotic resistance genes. To understand the evolutionary relationships among these bacterial genomes, we conducted a phylogenetic tree analysis, revealing the placement of the sequenced genomes within different bacterial groups. This analysis provided confirmation about the genetic relatedness and evolutionary history of the studied bacteria.

Overall, this study highlighted the complexity of antibiotic resistance patterns among different bacterial strains and emphasized the importance of genomic analysis to better understand the mechanisms underlying resistance.

Conclusion:-

In conclusion, this study has addressed critical healthcare concerns by identifying and characterizing bacterial strains responsible for Catheter-Related Bloodstream Infections (CRBSI) in Chronic Kidney patients. It filled an important gap in research, as there are relatively few studies that have focused on this clinical topic in Egypt. Traditional microbial culturing has its limitations, particularly when dealing with small, slow-growing, or antibiotic-treated bacteria. However, this study utilized the power of 16S rRNA gene sequencing, successfully identifying five bacterial isolates including *Enterococcusfaecalis*, *Staphylococcusaureus*, *Bacilluscereus*, and *Staphylococcushaemolyticus*, validating the efficacy of 16S rRNA sequencing in enhancing bacterial identification. Notably, the study also uncovered the presence of unusual bacteria like *Delftiatsuruhatensis*, which are rarely detected by conventional methods in CRBSI cases. Furthermore, antimicrobial sensitivity and susceptibility tests were conducted on the isolated bacterial strains. The results unveiled diverse responses, with some bacteria displaying multidrug resistance. This study reports novel whole-genomicfeatures and antimicrobial susceptibility patterns of clinical*Delftiatsuruhatensis* and *Enterococcusfaecalis* with a fully sequenced genome. Comparativeanalysis of *Delftia*and *Enterococcus*genomes provided a better understanding ofgenome divergence among strains of these genera. Furtherresearch should explore the epidemiological characteristics of*Delftiatsuruhatensis* and *Enterococcusfaecalis*, which may provide useful information for theclinical control of these bacteria.Overall, this research contributes significantly to our understanding of CRBSI and antibiotic resistance, offering valuable insights that can inform more effective treatment strategies and ultimately improve patient care in this critical healthcare domain.

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