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Phenotypic and Genotypic Identification of MDR Bacteria Isolated from the environment of ICU and Emergency Services in some Hospitals in benghazi, Libya .

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ABSTRACT

Microbial contamination in the hospital environment is one of the most important problems effecting the quality at health care provided there. This study aimed to determine the phenotypic and genotypic composition of MDR bacteria isolated from the environment of ICU andEMR in some benghazi hospital.among 100samples, with 50samles from each hospital. Results showed a bacterial contamination rate of 87% across all isolates. With Al-jalaa hospital recording the highest rate 90%. Gram negative bacteria accounted for 65.16% of isolates, while Gram positive 34.84% At the children hospital, the contamination rate was 84%, with Gram-Negative bacteria making up 52.63% of isolates. MRSA showed the highest contamination rate at Al-jalaa hospital, reaching 100% ,followed by Proteus mirabilis at 50% The lowest contamination rates were for P.chlororaphis at 18% and Achromobacter at 9%. At the children hospital, the highest contamination rate was for Achromobacter 75%, followed by S.aureus at 50% , MRSA at 36%, and K.pneumoniae at 25%. Four bacterial isolates which showed high resistance to different antibiotics (MDR) were re-identified by Molecular technique at the laboratory in of Molecular Biology, faculty of Science, University of Tunis, the 16S rRNA gene sequencing analysis revealed a high degree of similarity between the studied isolates and the reference genome. It was determined that all the isolates possess a genetic sequence similar to the reference genome. The results proved that the investigated ICU rooms at children hospital was contaminated with S.hemolyticus strain HaKim 1980, and K.pneumoniae strain YU-2304. While ICU at Al-jalaa hospital was contaminated with P.aeruginosa strain 2023CK-00050. Also, Emergency department at Al-jalaa hospital was contaminated with A.baumannii strain 2023CK-00001.

INTRODUCTION

" Nosocomial " term is used for any disease acquired by patient under medical care (Drachman, 1981). It is an infection acquired by patient during hospital stay. And it accounts for a major risk factor for serious health issues leading to death (Brusaferro et al., 2015). Nosocomial infections are caused by many microbes and each one can cause infection in health care settings, bacteria are responsible for about ninety percent infections . The distribution of bacteria in nosocomial infections is changing over time. Klebsiella spp., Proteus spp., and E-coli were responsible for nosocomial infections in the 1960s , but from 1975 to 1980s , Acintobacter spp. With P.aeruginosa created clinical difficulties (Kollef et al., 2021) Studies conducted in different parts of the world show, in North America and Europe 5%-10% of all hospitalization results in nosocomial infections, while Latin America, Africa and Asia show more than 40% hospitalization with nosocomial infections (Organization, 2002). Nosocomial infections accounts for 7% in developed and 10% in developing countries. According to WHO estimates, approximately 15% of all hospitalized patients suffer from these infections (Khan et al., 2017). Hospital acquired infection was reported in 3037 patients among 22.170 hospitalized patients in four hospital in Libya. Also, the highest incidence was in Benghazi Medical Center (17.9%)(Daw et al., 2023) The world health organization has recently declared antimicrobial resistance as one of the top ten global bublic health threats ,the process by which a strain of bacteria attains resistance to an antibiotic is an essentially complex.the reasons for attainment of antibiotic resistance vary from factors associated with the natural evolutionary process of the bacteria to bad health care environment. The former includes events like genetic mutation and selective pressure and the latter includes inadequate, inappropriate or veruse of antibiotics and poor hospital environment (Kumar, 2021).

This study aimed to Isolation and identification of bacteria from the environment (inanimate surfaces and air) and Assessment to the status of MDR bacteria and Genetic re-identification of MDR bacteria isolated from hospital environment inside ICU and emergency rooms at Al-Jalaa and benghazi Children hospitals.

METHODS

This study was condected from December 2022 to March 2023 in Aljalaa hospital and Benghazi Children hospital in Benghazi city, Libya. After wearing a sterile surgical gown, a total of 100 samples were taken using sterile cotton swabs from within the environment of the intensive care unit ICU, and emergency rooms include the instrument

table, floors,serum holder, the gloves of medical staff,beds,. As for isolating bacteria from the air,petri dishes containing the nutrient medium will be placed inside the intensive care unit and emergency rooms for an hourThese swabs were transported in sterile tubes containing the nutrient broth medium sterilized and transferred immediately to the microbiology laboratory, then inoculated on the blood agar and selective medium Maconkey agar , chocolate agar ,and manitol salt agar by streaking method and placed in the incubator for 24h at 37C. standard microbiology techniques were used after icubation. The bacterial culture characteristic(colony morphology on cultured plates , hemolysis, pigmentation, lactose fermentation) were tested. Biochemical tests were employed to conifirm bacterial identification , including coagulase test, catalase test, oxidase test ,urease test , triple sugar iron test. Antimicrobial susceptibility testing amodified kirby Bauer disk diffusion method was used to test each isolates .antimicrobial disks Gentamycin10mg,Levofloxacin 5mg,Tobramycin 10mg,Ceftazidime 30 mg, Sulphamethoxazole25 mg,Ciprofloxacin 30 mg, Ceftriaxone 30 mg, Cefepime 30 mg, Amikacin 25mg, Cefotaxime 30 mg,Augmentin 30mg,

Oxacillin10 mg. were aplied on nutrient agar plates and icubation at 37Co . the inhibition zones were measured then iterpreted as senstive , intermediate sestive or resistant per the standard criteria.

Molecular identification

Molecular identification was carried out by storage of isolates in nutrient broth media with glycerol and stored in -20Co then transported in ice bag to isolation of genomic DNA of the isolated bacterial colonies was extracted using boiling method following manufacturers guideline in laboratory of molecular biology in science college – Tunis Al-manar university .

The bacterial DNA was extracted using the boiling method, where the sample was heated at a high temperature to break the cell walls and release the DNA. The extract was then purified to remove any impurities that might interfere with the analysis and The 16S rRNA gene was amplified using specific primers through the PCR technique. The reaction conditions were optimized to ensure a clear and pure DNA product for sequencing.

Gene sequencing and Data Analysis, The PCR product was purified and subjected to sanger sequencing to obtain the full genetic sequence of the 16S rRNA gene from the sample.Sequencing data were analysed by Chromas Lit 2.4 software to identify the target sequence by alignment with the reference sequence. The obtained sequence

was subjucted to further analysis using Basic Local Alignment Search Tool (BLAST) for finding sequence similarity with sequences already reported in online database .

RESULTS

In this study the number of bacterial isolates was 100 samples.Bacterial growth was observed in 87% and 13% did not show any bacterial growth. the highest percent of contamination was observed at aljalla hospital reach to 90%. The bacterial contamination was higher with Gram- negative bacteria reach to 65.15%, while Gram- positive bacteria was 34.84%.while the percent of contamination was observed At benghazi Children hospital reach to 84%Table (1).The bacterial contamination was higher with gram negative bacteria reach to 52.6%, while gram positive bacteria was 47.3% table1. This study showed that among Gram-positive bacteria isolated in both hospitals, MRSA showed similar rate of contamination reach to 15%.However both S.aureus and M.varians showed higher percent of contamination in Children hospital reach to 14% and 15.7% respictively. Similar rate of contamination with S.pyogen was observed in both hospital. High rate of contamination was observed in Al-jalaa hospital with different genus of Gram-negative bacteria such as P.chlororaphis (18%) , followed by A.baumannii(10.6%), Achromobacter and Serratia (9%), Proteus mirabilis (6%), and P.psychrobacter (4.5%). whoever, in children hospital high rate of contamination was observed by Achromobacter (24%), P.psychrobacter (19%). K.pneumoniae observed only in Children hospital, the rate of contamination reach to 8.7%. figur(1).

The samples listed in the table (2) were isolated from various surfaces within ICU and emergency environments. These sources include air from emergency and ICU, serum holder in Emergency service at Al-jalaa hospital. Also, ventilator and gloves used in ICU at benghazi children hospital. The identified bacteria include K.pneumoniae., A.baumannii., MRSA.,and P.aeruginosa. preliminary analyses revealed significant resistance in K.pneumoniae ,. A.baumannii., MRSA,. P.aeruginosa samples to most antibiotics listed in the table(3), indicating that these strains exhibit multiple resistance mechanisms. Based on these findings, the samples were sent to a specialized laboratory in Tunisia for genomic analysis. The purpose of this procedure is to determine the precis genetic composition of these strains.

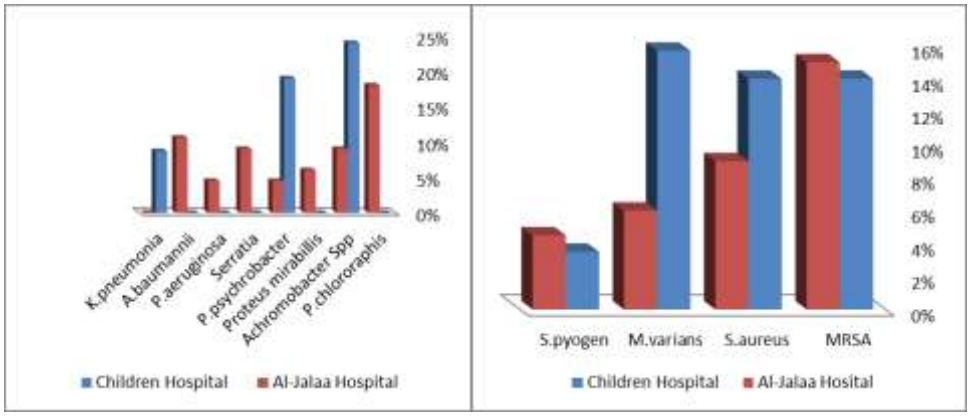
Bacterial identification using 16s rRNA sequencing

The use of 16s rRNA gene sequences is to identify specific genus and species for isolates and to confirm the isolates identification which were done by traditional methods. The 16s rRNA was detected in four isolates by PCR

method. In addition, Basic Local Alignment Search Tool (BLAST) revealed that all selective isolates were harbored the 16s rRNA gene and they were confirmed as the following Staphylococcus hemolytic (92%, Accession MT622589.1) (fig 2), P.aeruginosa (97.87%, Accession OR793893.1) (fig 3), K.pneumonia (99.18%, Accession OR789809) (fig 4), and Acintobacter baumannii(99.37%, Accession CP137927.1)(fig 5).Staphylococcus hemolytic was initially identified as methicillin resistant staphylococcus aureus (MRSA) based on phenotypic characteristics, including yellow pigmentation, methicillin resistance, and a negative Coagulase test. However, genomic analyses using sequencing techniques revealed that the isolate belongs to staphylococcus hemolytic , a species within the staphylococcus genus known for its ability acquire resistance genes. The presence of the mecA gene , responsible for methicillin resistance.was confirmed , explaining the observed resistance phenotype.table (4).

Results of culture	*Type of hospital*			
	Aljalla hospital		*Benghazi Children hospital*	
	No.	*%*	*No.*	*%*
No growth	*5*	*10%*	*8*	*16%*
Growth	*45*	*90 %*	*42*	*84%*
Total	*50*	*100 %*	*50*	*100 %*

Table 1: Distribution of bacterial Distribution of bacterial samples from Al-Jalaa Hospital and Benghazi Children Hospital.



(a) (b)

Figure1.Distribution of bacteria among Al-Jalaa Hospital and benghazi Children Hospital. a (Gram-negative) ,b(Gram- positive)

Table(2): Source of resistant bacteria in Al-jalaa and Children hospitals

Bacteria	Hospita	Department	Source of sample
MRSA	Benghazi Children hospital	ICU	Gloves
K.pneumoniae	Benghazi Children hospital	ICU	Ventilator
A.baumannii	Al-jalaa hospital	Emergency rooms	Serum holder
P.aeruginosa	Al-jalaa hospital	ICU	Air

Table (3): Affective antibiotics used against selected bacteria

Bacteria	Antibiotic used											
	CAZ	CN	TOB	ATM	TPZ	MEM	LEV	IPM	AK	FEP	CIP	CT
MRSA	R	R	R	R	S	R	S	R	R	R	R	R
K.pneumoniae	R	R	R	S	R	R	R	R	R	R	R	R
A.baumannii	R	R	R	S	S	R	R	R	R	R	S	R
P.aeruginosa	R	R	I	S	S	R	R	R	R	R	R	S

Table (4): Genotypic Identification of MDR bacteria isolated from Al-jalaa and Children hospitals.

Bacteria	Hospital	Department	Source of sample
S.hemolytic HaKim 1980	Benghazi Children hospital	ICU	Gloves
K.pneumoniae YU-2304	Benghazi Children hospital	ICU	Ventilator
A.baumannii 2023CK-00001	Al-jalaa hospital	Emergency rooms	Serum holder
P.aeruginosa 2023CK-00050	Al-jalaa hospital	ICU	Air

Figure2: Similarity between 16 r RNA gene sequence of strai staphylococcus aureus and other related species strain based on BLAST

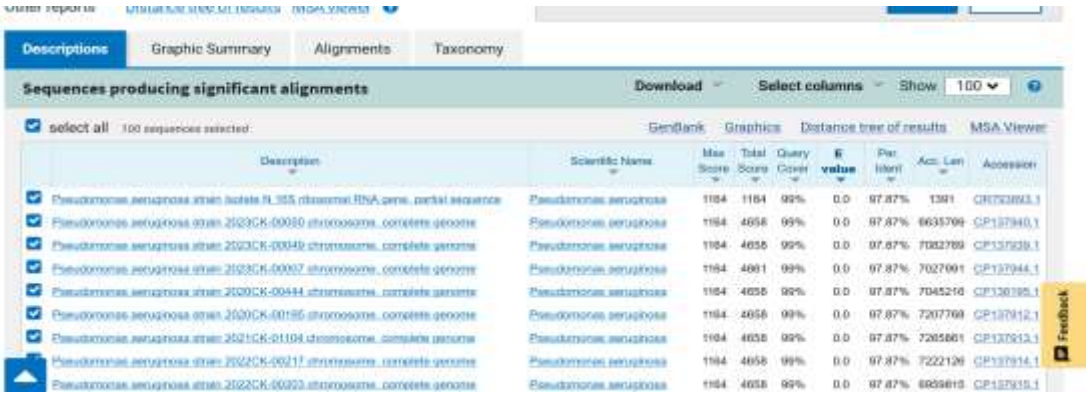


Figure3: Similarity between 16 r RNA gene sequence of strain Pseudomonas aeruginosa and other related species strain based on BLAST

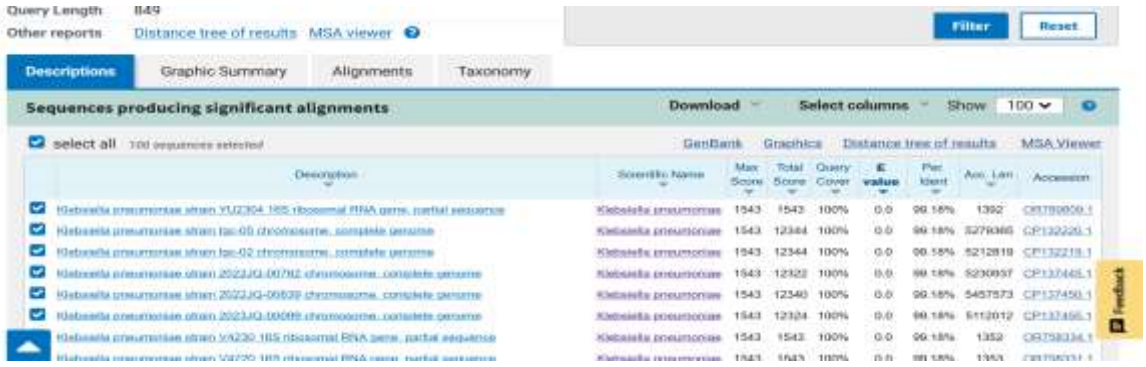


Figure4: Similarity between 16 r RNA gene sequence of strain K.pneumoniae and other related species strain based on BLAST.

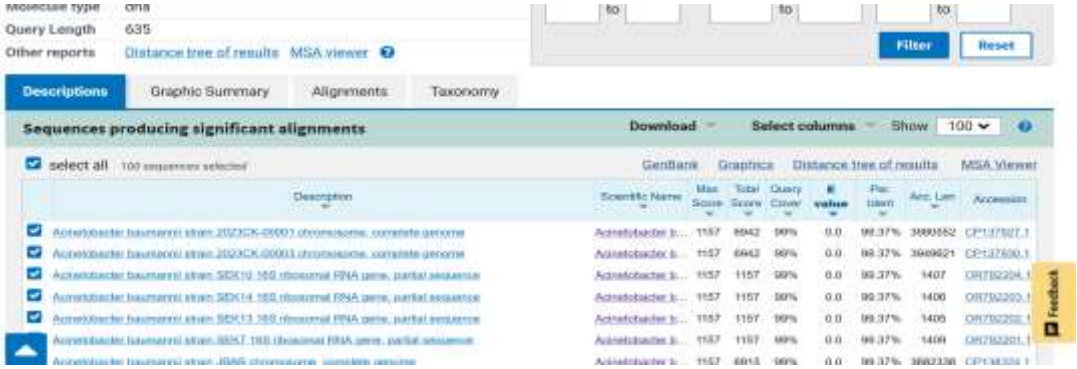


Figure5: Similarity between 16 r RNA gene sequence of strain A.baumannii and other related species strain based on BLAST.

DISCUSSION

The total of 100 samples evaluated, 87 sample showed positive bacterial growth . In Al-jalaa hospital bacterial growth reach to 90% . whoever the growth in benghazi Children hospital reach to 84%. Also, this study showed the prevalence of Gram- negative bacteria in Al-jalaa hospital reach to (65.1%) , while Gram- positive reach to (34.8%). Whereas, at children hospital the prevalence of Gram negative reach to (52%) , while Gram positive (47.3%). Similar results by (Chaoui et al 2019), who found that Gram negative reach to (51%), Gram positive (48.5%). The present study showed the antibiotic sensitivity of bacteria isolated from Al-jalaa hospital, all isolated were resistant to Ceftazidime followed by Ceftriaxone, Sulphamethoxazole , cefotaxime , imipenem, meropenem ,Azithromycin, Amikacin, Augmentin, Cephalexin, Oxaciliin. Similar results reported by (Effah et al 2020) who found that the highly resistant to Amikacin ,Ceftazidime, Ciprofloxacin. However, the antibiotic sensitivity of bacteria isolated from Emergency rooms at al-jalaa hospital, showed the resistant of bacteria to Oxacillin were (50%). Highly resistant to Oxacillin followed by tazobactam, Cefotaxime, Ceftriaxone, Imipenem, Ceftazidime, Amikacin, Augmentin , Gentamicin, Ciprofloxacin. In contrast, by (Ibrahim et al., 2024) who found high resistance rate to Ampicillin (100%), Oxacillin (95%), among Gram negative found high resistance to Ceftazidime (96.5%), Gentamycin (95%).In this study, we selected four highly antibiotic resistant bacterial isolates for genomic characterization using 16S rRNA sequencing. The isolates included MRSA K,pneumoniae,. A.baumannii,. P.aeruginosa. the results obtained using 16S rRNA sequencing highlight the significance of this technique in accurately identifying bacterial isolates at the genus and species levels. PCR successfully detected the 16S rRNA gene in all four isolates, and the use of the Basic Local Alignment Search Tool (BLAST) confirmed the genetic identity of the isolates with high accuracy, emphasizing its reliability compared to traditional methods. The first isolate was identified as P.aeruginosa with 97.87% similarity (Accession OR793893.1), aligning with its well-known classification and its association with antimicrobial resistance. The second isolate, K.pneumoniae, demonstrated a 99.18% similarity (Accession OR789809), confirming precise classification and emphasizing its role as a common pathogen in hospital aquired infections. the third isolate, A.baumannii, exhibited the highest similarity of 99.37% (Accession CP137927.1), indicating a highly accurate identification of this species , which is notorious for its multidrug resistance.the fourth isolate, Staphylococcus hemolytic, was identified with 92% similarity (Accession MT622589.1). while this confirms the species, the lower similarity compared to the other isolates may indicate minor genetic variations or mutations in the sequence.

Staphylococcus hemolytic was initially identified as methicillin resistant staphylococcus aureus (MRSA) based on phenotypic characteristics, However, genotypic analysis results showed that the strain was actually staphylococcus hemolytic, another species of the genus staphylococcus, which had acquired the mecA gene responsible for methicillin resistance.both MRSA and S.hemolytic can carry the mecA gene, which confer resistance to methicillin and related antibiotics. This similarity can result in confusion during traditional diagnostic

methods like antibiotic susceptibility testing. Similar results was reported by(Xu et al 2018) who found that S.hemolytic can carry methicillin-resistance genes such as mecA, which can lead to misidentification as MRSA when only clinical or antibiotic resistance tests are used. However , genetic diagnostics using methods like PCR or BLAST can accurately differentiate between these spices . similar results was observed by(Ruzauskas et al 2014) highlighted the importance of molecular diagnostics because S.hemolytic may exhibit methicillin resistance referred to as MRSH, but it differs biologically and clinically from MRSA. This underscores the necessity of using molecular tools, especially when analyzing samples from non-clinical or community settings.

This finding highlights the importance of combining phenotypic and genotypic analysis to ensure accurate bacterial classification particularly in the contex of antimicrobial resistance, which poses significant challenges in clinical settings

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