Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(3): 2015-2024. **OPEN ACCESS**

Identification of multidrug resistant bacterial isolates from Egyptian hospitals environments and molecular detection of their resistance genes

Abeer F. Abu Eleneen [1]*, Mohamed F. Ghaly[1] and Ahmed Shahin[2]

[1]Botany and microbiology department, Faculty of science, Zagazig University, **Egypt**
[2]Microbiology department, Faculty of medicine, Zagazig University, **Egypt**

***Correspondence:** Abfa112030@gmail.com **Received 30-104-2020, Revised: 20-07-2020, Accepted: 01-08-2020 e-Published: 10-08-2020**

Elevated infection incidence related to multidrug resistant (MDR) bacteria become a primary clinical importance. Thus, there is a need to isolate and identify MDR bacteria types and their antibiotics resistance mechanisms.Molecular identification of MDR bacterial isolates from Egyptian hospital's environment and to determine their antibiotic resistance genes (ARGs).MDR bacterial isolates were identified using antibiotic susceptibility test and 16s rRNA sequencing. ARGs were detected using multiplex PCR.The most effective antibiotic was imipenem and the most resistant one was oxacillin.Identified MDR bacteria were *Escherichia coli* (31%)*, Pseudomonas aeruginosa* (21.4%), *Staphylococcus aureus* (16.7%), *Klebsiella pneumonia* (14.3%)*, Enterococcus faecalis* (9.5%) and *Proteus vulgaris* (7.1%). The identification was confirmed by 16s RNA and Zero e-values were obtained for all queries which indicated that all alignments were significant with high query coverage values (99-100%) and high identities percent (98-99%). Among tested ARGs, *mecA* was found in *E. faecalis* and *S. aureus*. *vanA* was detected in *E. faecalis*. *KPC* was detected in *S. aureus, E. coli, P*. *aeruginosa* and *K. pneumonia. NDM-1* was detected in *P. aeruginosa* and *OXA-48* was detected in all tested isolates except *E. faecalis. qnrA* was detected in *S. aureus* and *K. pneumonia*. Finally, *qnrB* was detected in *P. vulgaris, E. faecalis, E. coli,* and *P. aeruginosa*. Hospital environments are considered MDR bacteria hot spots and this suggests that considerable precaution should be taken at all stages of the health care system to minimize MDR bacteria transmission among patients and medical staff.

Keywords: Multidrug resistant bacteria, Hospital environments, Molecular identification, 16s rRNA, Resistance genes, PCR

INTRODUCTION

In hospitalized patients, nosocomial infections or hospital-acquired infections affect the clinical outcomes and represent a serious concern worldwide (Wang et al. 2019). Both antibiotics overuse and misuse has led to the evolution of multidrug-resistant (MDR) bacteria, which now are considered as nosocomial infections major cause (Antony and Parija, 2016). In the public health, this antimicrobial resistance represents one of the most important global challenges. By the year 2050, it has been estimated that deaths related to infectious diseases-related complications will increase to about 10 million/year (Fuentes et al.

2019). Thus, it is crucial for effective treatment to understand the distribution, prevalence and clinical characteristics of nosocomial infections caused by MDR bacteria (Wang et al.2019).

Although MDR Gram-negative or positive bacteria are commonly defined as 'resistant to 3 or more antimicrobial categories'(Magiorakos et al. 2012), more accurate MDR definition has been proposed as 'non-susceptibility (non-susceptible, intermediate or resistant) to at least 1 agent in 3 or more antimicrobial classes (Wang et al., 2019). Several pathogenic bacteria that contributed significantly to global burden of infectious disease have presently developed MDR including *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumonia and pyogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella*, *Acinetobacter* and *Mycobacterium tuberculosis* (Fair and Tor, 2014). The frontier concern is that after drug development, bacteria take a very short period to develop resistance to it as a result of bacterial short generation time, response to environmental stimuli and resistance genes horizontal sharing between bacterial species (Mthembu et al., 2019). Thus, molecular identification of antibiotic resistance genes in bacterial pathogens by molecular methods is very important (Mthembu et al., 2019).

In clinical laboratories, multiplex polymerase chain reaction (PCR) for timely and accurate detection of resistance markers of MDR organism will help the clinicians in making early antibiotic adjustments. To give the result, it takes only about six hours compared to at least two days for antibiotic sensitivity test for result completion (Rathore et al., 2018). Also, 16S ribosomal RNA gene would be created to reflect bacterial diversity and it remains the primary reference for bacterial classification (Ntushelo, 2013). The current study aimed to determine the prevalence of MDR bacteria of isolates from Egyptian hospitals environments and its antimicrobial susceptibility profiles. Also, we aimed to identify the most resistant isolates using 16s rRNA and to detect resistance determinants or genes of the isolated bacteria species using multiplex PCR.

MATERIALS AND METHODS

Collection of clinical isolates

During the period of January-August/2016, clinical bacterial isolates (*n* =100) were derived from environments (air, water, floor, walls and equipment's) of Zagazig university hospitals, Zagazig, Egypt and from different medical patient's specimens. Informed consent was obtained from all individual participants included in the study. According to Murray et al. (Murray and Baron, 2007), the collected isolates were quickly transported under aseptic conditions to Microbiology Laboratory, Faculty of science, Zagazig University. Bacterial strains were streaked on ordinary nutrient agar medium and cultured at 37°C for several consecutive times until pure single colonies were obtained. Bacteria are classified on the basis of colony morphology, Gram staining, hemolysis on blood agar, biochemical reactions, and organism's enzyme activities.

Antibiotic susceptibility test

Using disk diffusion assays (Bauer et al., 1966) according to Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute, 2019), sensitivity of bacterial strains was determined for the following 14 antibiotics and antibiotic combinations (Oxoid Ltd., UK): imipenem, amikacin, ofloxacin, ciprofloxacin, nitrofurantoin, vancomycin, ceftriaxone, azithromycin, amoxicillin/clavulanic acid, cefaclor, amoxycillin, oxacillin, sulphamethoxazole/trimethoprim, and cephalothin. MDR was defined as an acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

DNA extraction, amplification, and 16S rRNA sequencing

DNA extraction was performed using GenElute™ bacterial genomic DNA kit (Sigma, USA) following the manufacturer's instructions. The 16S rRNA genes of the extracted DNA from each isolate were amplified with the universal primers namely 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3'), which has bacterial 16S rRNA gene conserved regions. Amplification was carried out in 25µL reaction volumes (adjusted to 25µL with sterile ultrapure water) containing the following: 2.5µL 10X PCR reaction buffer (100mM Tris-HCl, pH 8.3, 500mM KCl), 1.5µL 25mM $MgCl_2$ solution, 4.0µL 1.25mM, dNTPs, 0.5µL of 8F primer (200 ng/µL), 0.5µL of 1492R primer (200 ng/µL), 0.1µL AmpliTaq Gold DNA polymerase, and 1µL of DNA as template. PCR thermal cycling involved initial denaturation (94°C/3 minutes), 30 denaturation cycles (94◦C/1 minute), annealing (57◦C/1 minute), extension (72◦C/2 minutes), and a final extension (72◦C/8 minutes). To prevent false positive (reagents contamination) result, double distilled water was

used as negative control. At low constant voltage (10 V/cm), amplified products were separated in 1.0 % agarose gels in 1X Tris, Borate and EDTA buffer for 30 minutes. Separated fragments were extracted from the gel using Qiagen Gel purification kit and sequenced used 3130X DNA Sequencer (Genetic Analyzer, Applied Biosystems). For each isolate, the resulting sequences were compared to published sequences in Gen Bank using the Basic Local Alignment Search Tool (BLAST), http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Multiplex PCR for antibiotic resistance genes

Using multiplex PCR, antibiotic resistance genes including *mecA, vanA*, *vanB*, *KPC, NDM-1, OXA-48*, *qnrA* and *qnrB* were detected in the most resistant isolates. The sequence and amplicon size of the oligonucleotide primers chosen for amplification of the selected genes are shown in Table 1. The PCR reaction mixture contained 0.5µL DNA (50 ng) in 24.5µL Multiplex PCR Master Mix (QIAGEN®, Germany), 5 µL 10x primer mix, and 2 µM of each primer (Midland Certified Reagent Company Inc, USA). Each PCR program started with an initial heat-activation step at 95 °C for 15 min to activate HotStarTaq DNA Polymerase, then thermal cycling. At low constant voltage (70 V), amplified products (10µL of each mixed with 5µL of loading dye) were separated in 1.5% agarose gels in 1X Tris, acetate and EDTA buffer for 1 hour. The PCR DNA ladder was also applied. The gel was then placed in ethidium bromide for at least 30 minutes. The developed gels were then photographed and analyzed.

Table 1: Primers of resistance genes for PCR amplification

Group	Reference	Target gene	Primer sequence (5'-3')	Product size (bp)
Methicillin resistance genes	**(Rengaraj et al., 2016)**	***mecA***	F= ATCGATGGTAAAGGTTGGC R= AGTTCTGCAGTACCGGATTTGC	530bp
Glycopeptide resistance genes	**(Rengaraj et al., 2016)**	***vanA***	F= GCTATTCAGCTGTACTC R= CAGCGGCCATCATACGG	783bp
		vanB	F=CATCGCCGTCCCCGAATTTCAA R= GATGCGGAAGATACCGTCGCT	297bp
Serine β-lactamases class A **(Poirel *et al.,* 2011)**	**(Poirel et al., 2011)**	***KPC***	F= CGTCTAGTTCTGCTGTCTTG R= CTTGTCATCCTTGTTAGGCG	798bp
Serine β-lactamases class D	**(Poirel et al., 2011)**	***OXA-48***	F= GCGTGGTTAAGGATGAACAC R= CATCAAGTTCAACCCAACCG	483bp
Metallo β-lactamases class B	**(Poirel et al., 2011)**	***NDM-1***	F= GGTTTGGCGATCTGGTTTTC R= CGGAATGGCTCATCACGATC	621bp
Plasmid-mediated quinolone resistance (PMQR) genes	**(Robicsek et al., 2006)**	***qnrA***	F= ATTTCTCACGCCAGGATTTG R= GATCGGCAAAGGTTAGGTCA	516bp
	(Robicsek et al., 2006)	***qnrB***	F= GATCGTGAAAGCCAGAAAGG R= ACGATGCCTGGTAGTTGTCC	469bp

RESULTS

Distribution of collected isolates

From all bacterial isolates (n=100), 61 were gram negative and 39 were gram positive bacteria. The Gram negative bacterial isolates were isolated from urine samples of patients with urinary tract infections 29/61 (47.5%), pus of wound infections 9/61 (14.8%), sputum of respiratory tract infections 6/61 (9.8%), blood of blood infections 5/61 (8.2%) and hospital environments 12/61 (19.7%). The Gram positive bacterial isolates were isolated from urine samples of patients with urinary tract infections 5/39 (12.8%), pus of wound infections 17/39 (43.6%), sputum of respiratory tract infections 10/39 (25.6%), blood of blood infections 3/39 (7.7%) and hospital environments 4/39 (10.3%).

Antibiotic susceptibility patterns

As shown in Table 2, most of the bacterial isolates were highly susceptible (89%) to imipenem. Thus, it represents the most effective antibiotic. This was followed by amikacin, ofloxacin, ciprofloxacin, and nitrofurantoin with susceptibilities of 76, 67, 60 and 48%, respectively. On contrast, most of bacterial isolates (86%) were resistant to oxacillin followed by amoxycillin (75%), cephalothin (74%), and sulphamethoxazole/trimethoprim (69%). MDR bacterial isolates (42/100, 42%) were observed to be resistant against most groups (>60%) of antibiotics.

Identification of the multi-drug resistant isolates

To identify MDR bacteria, morphological, physiological, and biochemical analysis were

conducted (Table 3).

Table 2: Comparative susceptibility of bacterial isolates against different antibiotics

Antibiotic	Symbol	Conc. µg/disc	Resistant		Intermediate		Susceptible	
			No.	%	No.	%	No.	%
Imipenem	IPM	10	8	8	3	3	89	89
Amikacin	AK	30	14	14	10	10	76	76
Ofloxacin	OFX	5	32	32	1	1	67	67
Ciprofloxacin	CIP	5	32	32	8	8	60	60
Nitrofurantoin	F	300	44	44	8	8	48	48
Vancomycin	VA	30	53	53	4	4	43	43
Ceftriaxone	CRO	30	45	45	16	16	39	39
Azithromycin	AZM	15	62	62	2	2	36	36
Amoxicillin/clavulanic acid	AMC	30-20/10	62	62	9	9	29	29
Cefaclor	CEC	30	50	50	33	33	17	17
Amoxycillin	AX	25	75	75	11	11	14	14
Oxacillin	OX	1	86	86	5	5	9	9
Sulphamethoxazole/trimethoprim	SXT	23.8/1.25	69	69	24	24	7	7
Cephalothin	CL	30	74	74	21	21	5	5

Table 3: Biochemical tests and morphological characters of MDR isolates

Test	Results					
Gram's stain	**- ve**	**- ve**	**- ve**	**- ve**	**+ ve**	**+ ve**
Shape of cell	**Bacilli**	**Bacilli**	**Bacilli**	**Bacilli**	**Cocci**	**Cocci**
Arrangement	Short rods	Short rods	Short rods	Rods	Irregular clusters	Diplococci
Colonies characters	Smooth, convex, moist, translucent, gray with a shiny surface, entire edge and Pink colonies on MacConkey agar	Elevated and mucoid appearance, pink colonies on MacConkey agar	Swarm with Periodic cycles of migration producing concentric zones, or spread in a uniform film	large, smooth, with flat edges, elevated center and produce blue green pigment on nutrient agar	Raised, smooth, glistening, translucent, with entire margins. Pigmentation varies from gray to yellow to orange.	Pinpoint to small, smooth and entire edge
Motility	+ ve	- ve	+ ve	+ ve	- ve	- ve
Catalase	+ ve	+ ve	+ ve	+ ve	+ ve	- ve
Oxidase	- ve	- ve	- ve	+ ve	- ve	- ve
Coagulase	- ve	- ve	- ve	- ve	+ ve	- ve
Indole	+ ve	- ve	+ ve	- ve	- ve	- ve
Citrate	- ve	+ ve	- ve	+ ve	- ve	+ ve
MR	+ ve	- ve	+ ve	+ ve	+ ve	+ ve
VP	- ve	+ ve	- ve	- ve	+ ve	+ ve
H_2S production	- ve	- ve	+ ve	- ve	- ve	- ve
Urease	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
Nitrate reduction	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Blood hemolysis	γ-hemolysis	γ-hemolysis	α–hemolysis	β-hemolysis	β-hemolysis	γ–hemolysis
Gelatin liquification	- ve	- ve	+ ve	+ ve	- ve	- ve
Tellurite reduction	- ve	- ve	+ ve	- ve	+ ve	+ ve
Blue green pigment	- ve	- ve	- ve	+ ve	- ve	- ve
Sugars Fermentation						
D-Glucose	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Sucrose	+ ve	+ ve	+ ve	- ve	+ ve	+ ve
Lactose	+ ve	+ ve	- ve	- ve	+ ve	+ ve
D-Xylose	+ ve	+ ve	+ ve	- ve	- ve	- ve
D- sorbitol	+ ve	+ ve	- ve	- ve	- ve	- ve
D-Mannitol	+ ve	+ ve	- ve	+ ve	+ ve	+ ve
Identification	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*S. aureus*	*E. faecalis*

MR =Methyl red test; VP = Voges-Proskauer reaction; - ve = Negative; + ve = Positive.

Table 4:Blast results for 16S rRNA sequences from MDR isolates

Accession number	Reference number	Description	Querycoverage %	E-value	Identity %
KY421542	AFA28	*S. aureus*	100	0.0	99
KY421543	AFA31	*E. coli*	99	0.0	99
KY421544	AFA46	*P. aeruginosa*	100	0.0	99
KY421545	AFA65	*K. pneumonia*	100	0.0	99
MH429784	AFA13	*P. vulgaris*	100	0.0	98
MH429785	AFA22	*E. faecalis*	99	0.0	98

Table 5: Detection of antibiotic resistance genes using multiplex PCR

Bacteria	Resistance gens							
	mecA	***vanA***	***vanB***	***KPC***	***NDM-1***	***OXA-48***	***qnrA***	***qnrB***
P. vulgaris	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
E. faecalis	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve
S. aureus	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve
E. coli	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve
P. aeruginosa	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve
K. pneumoniae	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve

+ve= positive, -ve= negative

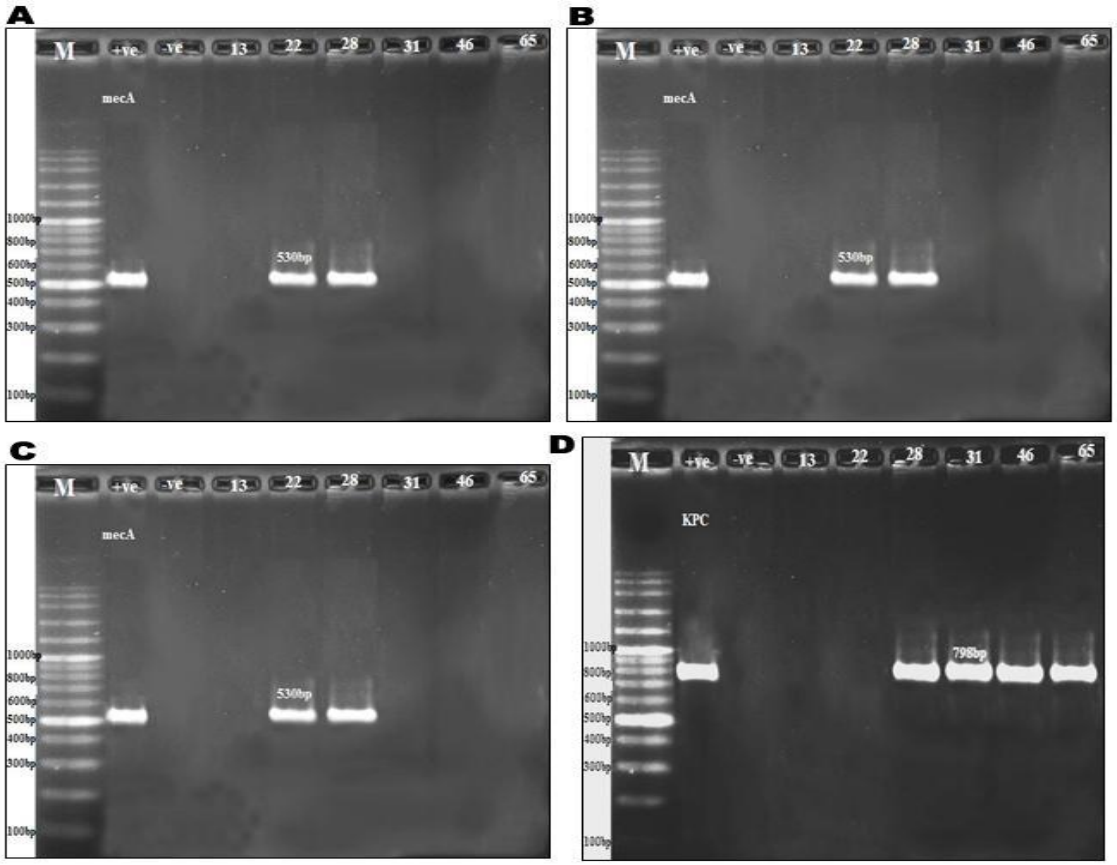

Figure 1. Gel electrophoresis of PCR amplicons of (A) *mecA,* (B) *vanA*, (C) *vanB* and (D) *KPC* genes. Lane M: Molecular marker, lane +ve: Positive control, lane -ve: Negative control, lanes 13, 22, 28, 31, 46 and 65: the tested isolates of *Proteus vulgaris, Enterococcus faecalis, S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae,* respectively.

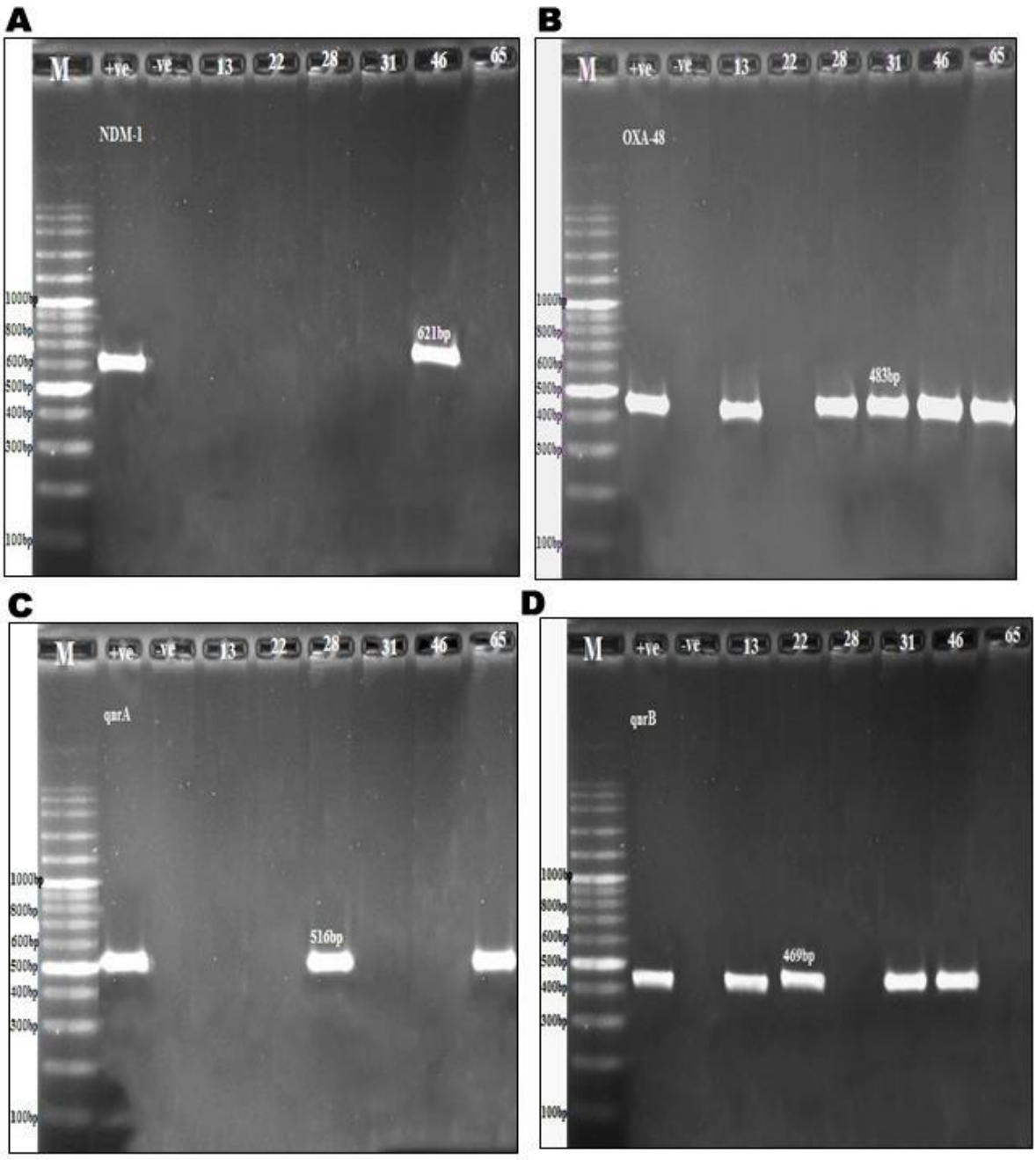

Figure 2. Gel electrophoresis of PCR amplicons of (A) *NDM-1,* (B) *OXA-48*, (C) *qnrA* and (D) *qnrB* genes. Lane M: Molecular marker, lane +ve: Positive control, lane -ve: Negative control, lanes 13, 22, 28, 31, 46 and 65: the tested isolates *Proteus vulgaris, Enterococcus faecalis, S. aureus, E. coli, P. aeruginosa* and *K. pneumonia,* respectively.

According to identification protocols, MDR bacterial isolates were divided into *Escherichia coli* (13/42, 31%), *Pseudomonas aeruginosa* (9/42, 21.4%), *Staphylococcus aureus* (7/42, 16.7%), *Klebsiella pneumonia* (6/42, 14.3%), *Enterococcus faecalis* (4/42, 9.5%) and *Proteus vulgaris* (3/42, 7.1%).

Identification of the most resistant bacterial isolates using 16S rRNA

Bacteria identification was confirmed using

16S rRNA gene sequencing. Sequences of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, P. vulgaris,* and *E. faecalis* were submitted to GenBank at the NCBI website (www.ncbi.nlm.nih.gov) as accession numbers KY421542, KY421543, KY421544, KY421545, MH429784, and MH429785, respectively.

The BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi?) and phylogenetic analysis using the Clustal W muscle algorithm program were used to assess the DNA similarities of the obtained 16S rRNA gene sequence (Table 4).

Detection of antibiotic resistance genes using multiplex PCR

Using multiplex PCR technique, antibiotic resistance genes (*mecA, vanA*, *vanB*, *KPC, NDM-1, OXA-48*, *qnrA,* and *qnrB*) were detected in the most resistant isolates by amplifying the respective genes. Findings are shown in Table 5 and Figures 1 and 2.

DISCUSSION

In clinical practice worldwide, infectious diseases related to antibiotic resistance development and spread is a major threat to human health. In any country, antibiotic resistance can affect anyone, of any age (Khalifa et al., 2019). In developing countries including Egypt, there are limited data regarding the distribution of different MDR bacteria categories, especially gram-negative bacteria, and their antimicrobial resistance gene determinants (Khalifa et al., 2019). Thus, there is a need to select and identify MDR bacteria to identify their types and their antibiotics resistance mechanisms (Abdel-Shafi, 2013).

In this study from one hundred clinical bacterial isolates derived from Egyptian hospital's environments and patients, there were 61% gram-negative and 39% gram-positive bacteria. On dry hospital surfaces, many microbiological studies reported that certain hospital pathogens can survive for extended periods (Otter et al., 2013). On dry inanimate surfaces, both gram-negative and -positive bacteria can survive up to months with longer persistence under humid condition and lower temperature (Kramer et al., 2006). As obtained in our results, predominance of gram-negative organisms may be commonly found in hospital's environment in Egypt (See et al., 2013) and other countries worldwide (Vincent et al., 2009; Doyle et al., 2011).

Here, isolates antibiotic resistance patterns were assessed and traditional antimicrobial susceptibility test revealed that the most effective antibiotic was imipenem followed by amikacin, ofloxacin, ciprofloxacin, and nitrofurantoin, respectively. Imipenem was previously reported to be the most effective and similar effectiveness of these antibiotics categories were also reported (Bahgat, 2015; Reddy, 2016). Overall MDR rate in this study was 42%. This result is consistent with results reported in other Egyptian related studies (Bahgat, 2015), but relatively lower than other reports in Egypt (Khalifa et al., 2019) and other African countries (Godebo et al., 2013; Dramowski et al., 2015; Feleke et al., 2018). These variations may be due to environmental, host and microbial factors and also due to different sample sources (Dusé, 2005). In this study, MDR bacterial isolates were *Escherichia coli* (31%) followed by *Pseudomonas aeruginosa* (21.4%), *Staphylococcus aureus* (16.7%), *Klebsiella pneumonia* (14.3%)*, Enterococcus faecalis* (9.5%) and *Proteus vulgaris* (7.1%). In hospitals, MDR *E. coli* are widely distributed and are increasingly being isolated from community (Ibrahim et al., 2012). Also, several reports reported significant increase in MDR *P. aeruginosa* isolates especially in intensive care units (Hirsch and Tam, 2010). In the hospital setting, *S. aureus* related to high bloodstream infections percent (20%) and its treatment is often challenging due to the emergence of MDR strains (Kadariya et al., 2019). Also, *K. pneumonia* is one of the leading MDR gram-negative isolates (Moini et al., 2015; Feleke et al., 2018). Studies have suggested that enterococci strains serve as reservoir for antibiotic resistance genes which can be transferred among enterococci or acquired by other bacteria (Adesida et al., 2017). Earlier studies found that *P. vulgaris* was resistant against most common used antibiotics (Mandal, 2015). Similar distribution of MDR bacteria spices obtained in this study was reported in different previous studies (Tantry and Rahiman, 2012).

Highly suitable 16S rRNA sequencing is a universal phylogenetic marker and it is useful bacterial classification method. In which for bacterial identification of the target samples, the nucleotide sequences target region are determined and compared with sequences available from databases to yield homology matches (Alsanie, 2018). In the current study, identification of MDR isolates was confirmed by 16S rRNA gene sequencing and Zero e-values were obtained for all 6 queries (*E. coli*, *P. aeruginosa, S. aureus*, *K. pneumonia*, *E. faecalis* and *P. vulgaris*) which

indicated that all alignments were significant. Also, high query coverage values (99-100%) and high percent identities (98-99%) were observed.

These types of antimicrobial resistance associated with genetic mutation and intra- or inter-species transfer of resistance gene (Munita and Arias, 2016). Thus, molecular identification of these resistance genes by molecular methods is very important (Mthembu et al., 2019). Application of multiplex PCR was reported as a useful tool to identify genes of resistance with high sensitivity and high throughput and rapid results (Rathore et al., 2018). In the current study, antibiotic resistance genes detected in *E. coli* were *KPC*, *OXA-48* and *qnrB*, in *P. vulgaris* were *OXA-48* and *qnrB*, in *E. faecalis* were *mecA*, *vanA* and *qnrB*, in *S. aureus* were *mecA*, *KPC*, *OXA-48* and *qnrA*, in *P. aeruginosa* were *KPC*, *NDM-1*, *OXA-48* and *qnrB* and in *K. pneumonia* were *KPC*, *OXA-48* and *qnrA*. During last decades, the pandemics caused by these genes-producing *Enterobacteriaceae* highlighted the desperate need for global surveillance systems of antimicrobial resistance. In resource-limited countries, these surveillance systems would help to assess antimicrobial resistance scope. Also, studies combining surveillance with molecular identification would help to detect emerging or novel resistance mechanisms in travelers returning from endemicity areas and help to curb antibiotic resistance worldwide spread (Lascols et al., 2013)..

CONCLUSION

Taken together, this study demonstrated the presence of important clinical bacterial strains in raw Egyptian hospital. High proportion of these strains showed antibiotic resistance. Some antimicrobial resistance genes were detected in these MDR bacteria that are known to confer resistance to antibiotics lines that are preferred for the treatment of Gram-positive and -negative infections. Thus, hospital environments are considered “hot spots” of antibiotic resistant bacteria and this suggests that considerable precaution should be taken at all stages of the health care system to minimize MDR bacteria transmission among patients and medical staff in both in- and out-patient settings in hospitals and community.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

AFA would like to thank Dr. Mohamed A. Abdelrazek for his help and involvement in writing the manuscript.

AUTHOR CONTRIBUTIONS

AFA performed all experiments. MFG and AS were chief investigators who conceptualised and designed the study design. AFA also wrote the manuscript. All authors read and approved the final version.

REFERENCES

References

Abdel-Shafi S, Ouda SM, Elbalat I, Enan G. (2013). Characterization and Identification of Multidrug Resistant Bacteria from Some Egyptian Patients. Biotechnology; 12:65-73.

Adesida SA, Ezenta CC, Adagbada AO, Aladesokan AA, Coker AO. (2017). Carriage of multidrug resistant enterococcus faecium and enterococcus faecalis among apparently healthy humans. African journal of infectious diseases; 11:83-89.

Alsanie WF, Felemban EM, Farid MA, Hassan MM, Sabry A, Gaber A. (2018). Molecular Identification and Phylogenetic Analysis of Multidrug-resistant Bacteria using 16S rDNA Sequencing. Journal of Pure and Applied Microbiology; 12:489-496.

Antony HA, Parija SC. (2016). Antimalarial drug resistance: An overview. Trop Parasitol; 6:30-41.

Bahgat MMM, Elbialy AA, Zaky MMM, Toubar SME. (2015). Prevalence of antibiotic resistant aerobic bacteria isolated from surgical wounds of inpatients at Zagazig University Hospitals, Egypt. Int J Curr Microbiol App Sci; 4:460–472.

Bauer AW, Kirby WM, Sherris JC, Turck M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am J Clin

Pathol; 45:493-496.
Clinical and Laboratory Standards Institute (2019). Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100. . In, P. Wayne, ed.
Doyle JS, Buising KL, Thursky KA, Worth LJ, Richards MJ. (2011). Epidemiology of infections acquired in intensive care units. Semin Respir Crit Care Med; 32:115-138.
Dramowski A, Cotton MF, Rabie H, Whitelaw A. (2015). Trends in paediatric bloodstream infections at a South African referral hospital. BMC Pediatr; 15:33.
Dusé AG. (2005). Infection control in developing countries withparticular emphasis on South Africa. Southern African Journal of Epidemiology and Infection; 20:37-41.
Fair RJ, Tor Y. (2014). Antibiotics and bacterial resistance in the 21st century. Perspectives in medicinal chemistry; 6:25-64.
Feleke T, Eshetie S, Dagnew M*, et al.* (2018). Multidrug-resistant bacterial isolates from patients suspected of nosocomial infections at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. BMC research notes; 11:602-602.
Fuentes MD, Gutierrez S, Sahagun D*, et al.* (2019). Assessment of Antibiotic Levels, Multi-Drug Resistant Bacteria and Genetic Biomarkers in the Waters of the Rio Grande River Between the United States-Mexico Border. Journal of health & pollution; 9:190912.
Godebo G, Kibru G, Tassew H. (2013). Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. Ann Clin Microbiol Antimicrob; 12:17.
Hirsch EB, Tam VH. (2010). Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. Expert review of pharmacoeconomics & outcomes research; 10:441-451.
Ibrahim ME, Bilal NE, Hamid ME. (2012). Increased multi-drug resistant Escherichia coli from hospitals in Khartoum state, Sudan. African health sciences; 12:368-375.
Kadariya J, Thapaliya D, Bhatta S*, et al.* (2019). Multidrug-resistant Staphylococcus aureus Colonization in Healthy Adults Is more Common in Bhutanese Refugees in Nepal than Those Resettled in Ohio. BioMed research international; 2019:5739247.
Khalifa HO, Soliman AM, Ahmed AM*, et al.* (2019). High Prevalence of Antimicrobial Resistance in Gram-Negative Bacteria Isolated from Clinical Settings in Egypt: Recalling for Judicious Use of Conventional Antimicrobials in Developing Nations. Microb Drug Resist; 25:371-385.
Kramer A, Schwebke I, Kampf G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis; 6:130.
Lascols C, Peirano G, Hackel M, Laupland KB, Pitout JDD. (2013). Surveillance and molecular epidemiology of Klebsiella pneumoniae isolates that produce carbapenemases: first report of OXA-48-like enzymes in North America. Antimicrobial agents and chemotherapy; 57:130-136.
Magiorakos AP, Srinivasan A, Carey RB*, et al.* (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect; 18:268-281.
Mandal DD, S. K.; Das, B.; Sengupta, M.; Kundu, P. K.; Roy, S. (2015). Isolation and characterization of multi-drug resistance proteus vulgaris from clinical samples of uti infected patients from midnapore, west bengal. IJLPR; 5:32-45.
Moini AS, Soltani B, Taghavi Ardakani A*, et al.* (2015). Multidrug-Resistant Escherichia coli and Klebsiella pneumoniae Isolated From Patients in Kashan, Iran. Jundishapur J Microbiol; 8:e27517.
Mthembu TP, Zishiri OT, El Zowalaty ME. (2019). Molecular Detection Of Multidrug-Resistant Salmonella Isolated From Livestock Production Systems In South Africa. Infect Drug Resist; 12:3537-3548.
Munita JM, Arias CA. (2016). Mechanisms of Antibiotic Resistance. Microbiology spectrum; 4:10.1128/microbiolspec.VMBF-0016-2015.
Murray PR, Baron EJ (2007). Manual of Clinical Microbiology. In, W. ASM Press, D.C., ed.
Ntushelo K. (2013). Identifying bacteria and studying bacterial diversity using the 16S ribosomal RNA gene-based sequencing techniques: A review. African journal of Microbiology research; 7:5533-5540.
Otter JA, Yezli S, Salkeld JA, French GL. (2013). Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. Am J Infect Control; 41:S6-11.
Poirel L, Walsh TR, Cuvillier V, Nordmann P. (2011). Multiplex PCR for detection of

acquired carbapenemase genes. Diagn Microbiol Infect Dis; 70:119-123.

Rathore K, Joseph B, Sharma DK*, et al.* (2018). Evaluation of multiplex polymerase chain reaction as an alternative to conventional antibiotic sensitivity test. Veterinary world; 11:474-479.

Reddy PS, John MS, Devi PV, Kumar SS. (2016). Nosocomial infections among patients admitted in general ICU: study from a tertiary-care hospital in South India. Int J Med Sci Pub Health; 5:21–27.

Rengaraj R, Mariappan S, Sekar U, Kamalanadhan A. (2016). Detection of Vancomycin Resistance among Enterococcus faecalis and Staphylococcus aureus. J Clin Diagn Res; 10:Dc04-06.

Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC. (2006). qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. Antimicrob Agents Chemother; 50:2872-2874.

See I, Lessa FC, ElAta OA*, et al.* (2013). Incidence and pathogen distribution of healthcare-associated infections in pilot hospitals in Egypt. Infection control and hospital epidemiology; 34:1281-1288.

Tantry BA, Rahiman S. (2012). Antibacterial resistance and trend of urinary tract pathogens to commonly used antibiotics in Kashmir Valley. West Indian Med J; 61:703-707.

Vincent JL, Rello J, Marshall J*, et al.* (2009). International study of the prevalence and outcomes of infection in intensive care units. Jama; 302:2323-2329.

Wang M, Wei H, Zhao Y*, et al.* (2019). Analysis of multidrug-resistant bacteria in 3223 patients with hospital-acquired infections (HAI) from a tertiary general hospital in China. Bosnian journal of basic medical sciences; 19:86-93