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Characterization of biofilm formation, pyocyanin production, and antibiotic resistance mechanisms in drug-resistant *Pseudomonas aeruginosa* isolated from children in Egypt

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ABSTRACT

Children can be exposed to more bacterial infections, which has become of importance, especially when bacteria resist many of the antibiotics used today. Twelve *Pseudomonas aeruginosa* strains were collected and identified. Antibiotic sensitivity testing, biofilm formation, and pyocyanin production were evaluated. A cluster analysis was performed. Phenotypic and genotypic detection of efflux pump activity and Metallo-β Lactamase (MβLs) resistance mechanisms were studied. A marked resistance (100%) resulted toward antibiotics including cefotaxime, cefoxitin, ceftriaxone, and meropenem. Imipenem and amikacin antibiotics showed sensitivity percentages of 41.7 and 33.3, respectively. 66.7% were able to form a biofilm at which they were categorized as moderate (25%) and weak (41.7%). Phenotypically, 41.67% of the isolates were MBL metallo-β-lactamase-positive, but genotypically, *bla*VIM gene was detected only in one isolate, while *bla*IMP was not detected in any isolate. MexR and MexZ genes were detected in all isolates (100%). NfxB gene and MexT gene were found in 27.27% and 45.45% of isolates, respectively. All the efflux genes were found collectively in three isolates. This study highlights the occurrence of antibiotic resistance, besides the production of important virulence factors (biofilm formation and pyocyanin production). Also, the gene occurrence of antibiotic resistance mechanisms was reported among our *P. aeruginosa*. This virulent bacterial behavior is alarming which needs attention to the way we use antibiotics.

INTRODUCTION

Pseudomonas aeruginosa is a pathogen that can survive in different environments (Logan *et al*., 2017). It is also described as an important human pathogen, responsible for nosocomial infections (Ruiz-roldán *et al*., 2018). It is reported that multidrug-resistant (MDR) *P. aeruginosa* infections were associated with cost increase per patient in comparison with those nonresistant ones (Morales *et al*., 2012). Children are much more sensitive to disease-causing bacteria than adults, whereas no descriptions exist about characterization of *P. aeruginosa* isolates from children (Ruiz-roldán *et al*., 2018).

However, the treatment of those infections has become of importance as a result of its antibiotic resistance behavior (Pachori *et al*., 2019). Nowadays, *P. aeruginosa* displays resistance to a variety of antibiotics, including different classes (Alnour and Ahmed-Abakur, 2017), besides a large number of virulence factors (toxin A, alkaline protease, elastase, exoenzymes, and pyocyanin production) that play important roles in the pathogenicity process (Jazayeri *et al*., 2016). Bacterial biofilm formation in both medical and industrial fields causes big problems, as these structures resist the antibiotics and biocides treatments, besides reducing the host immune-responses action (Lima *et al*., 2018). *Pseudomonas aeruginosa* intrinsic resistance includes efflux pump expression that expels antibacterial agents outside the cell and produces antibiotic inactivating enzymes (Sachdeva *et al*., 2017). Because of all this, this study was aimed at studying antibiotic resistance profile, resistance mechanisms, and virulence factors in *P. aeruginosa* collected from children.

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MATERIALS AND METHODS

Collection and identification *P. aeruginosa* strains

The study was conducted with 12 *P. aeruginosa* selected out of 136 *Pseudomonas* species that were collected from clinical samples routinely sent to the microbiology laboratory in a tertiary care pediatric Cairo University hospital in 3 months from October 2019 to December 2019. All the collected isolates were identified according to the standard procedures and confirmed by growing on Cetrimide agar and examined under ultraviolet to see the fluorescence of their colonies (Singh *et al*., 2015). The standard strain *P. aeruginosa* (American Type Culture Collection 15442) was used as control.

Antibiotics susceptibility tests

The bacterial antibiotic susceptibility profile was tested using Kirby–Bauer disk diffusion technique (Clinical and Laboratory Standards Institute (CLSI), 2019), using the commercial antibacterial agents (Oxoid, UK): ciprofloxacin (CIP, 5 µg), meropenem (MEM, 10 µg), gentamycin (CN, 10 µg), amikacin (AK, 30 µg), cefotaxime/cloxacillin (CTC, 40 µg), cefotaxime (CTX, 30 µg), cefoxitin (FOX, 30 µg), ceftriaxone (CRO, 30 µg), and cefepime (FEP, 30 µg).

Detection of virulence factors

Biofilm ability using microtiter plate assay

Biofilm formation

Pseudomonas aeruginosa isolates were screened for their biofilms formation ability by microtiter plate according to the method described (Mohamed *et al*., 2018c). 100 µl *P. aeruginosa* suspension of 0.5 McFarland was added to sterile brain heart infusion broth (100 µl) in wells of 96-well microtiter plate (Cellstar®, Greiner Bio-One) and then incubated at 37°C. The assay was done in triplicate.

Biofilm quantification using crystal violet (CV) assay

After 18 hours incubation period, wells were evacuated and gently washed. *Pseudomonas aeruginosa* biofilms formed on the walls were dried and stained with 1% CV for 20 minutes. After properly washing and drying, the stained *P. aeruginosa* biofilm was reconstituted in absolute ethanol, and its absorbance was measured at 630 nm using microplate reader (Stat Fax-2100; GMI, Inc.,). Optical density cut-off value (O.Dc) was calculated as mentioned by (Mohamed *et al*., 2020b).

Pyocyanin production

Phenotypic visual detection

Phenotypic detection was done as described with minor modifications. Briefly, the bacterial suspension (0.5 McFarland) was swabbed on *Pseudomonas*-Agar P media plates supplemented with 1.5% glycerol. After incubation for 24 hours, agar was cut into small pieces, and pyocyanin was extracted with chloroform after shaking. The chloroform color changed to dark green or blue and then was re-extracted into 1 ml of HCl (0.2N) to give a pink to a deep red solution, which was considered positive results (Feghali and Nawas, 2018).

Quantitative of pyocyanin

Pigment quantification was done depending on the absorbance of pyocyanin in acidic solution, at which solution was measured, and the concentrations expressed as µg/mL of pigment produced by the culture supernatant were calculated by using the optical density reading at 520 nm multiplying by 17.072 (Khadim and Marjani, 2019).

Cluster analysis

Based on the phenotypic traits like antibiotics susceptibility results and virulence factors (biofilm formation ability and pyocyanin production) of different *P. aeruginosa* strains, hierarchical cluster analysis was performed using Statistical Package for the Social Sciences software (SPSS Inc. v. 12). The antibiotic susceptibility results for each were coded as "1" resistant and "0" nonresistant. The positive results of biofilm formation and pyocyanin production for each strain were coded as "1" where the negative ones were coded "0." The analysis was presented graphically to find the similarity among strains, based on average linkage, and the branch length represents the distance between the strains (Mohamed *et al*., 2018a).

Phenotypic detection of efflux pump activity

Minimum inhibitory concentration (MIC)

Resistance to CIP was confirmed by MIC evaluation using an antibiotic agar dilution test (Al Rashed *et al*., 2020). CIP stock solution was prepared according to manufacturer recommendation. Antibiotic final concentration range of 2 mg/l up to 512 mg/l and 0.5 McFarland standard bacterial turbidity were used. A volume of 10 µl of each inoculum was pipetted into serial agar plates. After incubation for 18 hours at 37°C, MIC was read. The interpretation of MIC values was done according to CLSI recommendations (Clinical and Laboratory Standards Institute (CLSI), 2019).

Ethidium bromide-(EtBr-) agar cartwheel method

Tryptic soy agar plates containing EtBr concentrations (Sigma-Aldrich) of 0–3 mg/l were swabbed with bacterial suspension (0.5 MacFarland), making cartwheel pattern (Osman *et al*., 2018). All plates were examined under the UV light, after the incubation period of 18 hours at 37ºC. EtBr concentration at which the swabbed culture produces fluorescence was recorded. The higher the concentration of EtBr required for the appearance of fluorescence considered, the greater the EP activity. *P. aeruginosa* standard strain is considered a negative control.

Detection of MβLs

This test was done using the combined imipenem/ethylenediaminetetraacetic acid (EDTA) disk method (Alkhudhairy and Al-Shammari, 2020). Single imipenem disk and imipenem disk (10 µg) supplemented with 10 µl EDTA (750 µg) were placed on the plate and incubated for 18 hours period at 37°C. A 7 mm inhibition increase of the imipenem/EDTA disk than the single imipenem disk was considered a positive result.

Genotypic detection of resistance mechanisms

Total bacterial DNA extraction was done as mentioned by Mohamed and Khalil (2020). Detection of resistance genes was done using primers mentioned in Table 1. Cycling conditions for the efflux pump regulatory genes and both *bla*IMP and *bla*VIM genes were performed as mentioned. The amplified DNA was electrophoresed using 1.6% agarose gel stained with EtBr, and the bands were visualized under ultraviolet trans-illuminator (Alkhudhairy and Al-Shammari, 2020; Osman *et al*., 2018).

RESULTS

In this study, 12 *P. aeruginosa* strains were included, at which endotracheal aspirate (50%) was the most predominant source among our isolates, followed by sputum (33.33%) and urine (16.67%). High resistance rates (100%) toward cephems antibiotics (including CTX, FOX, and CRO) and carbapenems antibiotic (MEM) were observed (Table 2). On the other hand, imipenem and AK were the most effective antibiotics by sensitivity percentages of 41.7 and 33.3, respectively. Out of 12 strains, 8 (66.7%) were able to form a biofilm at which they were categorized as moderate (25%) and weak (41.7%); besides, 4 isolates (33.3%) were found non-biofilm formers. Three isolates showed positive results through the visual pyocyanin test (Table 3), and by spectrophotometer, pyocyanin concentrations of 1.23, 1.1, and 0.836 µg/ml were detected.

Cluster analysis of converted antimicrobial susceptibility and virulence factors production data was performed and presented in a dendrogram (Fig. 1) showing the difference between the used *P. aeruginosa* strains.

CIP susceptibility was confirmed among all isolates using agar dilution method, at which isolates were categorized at breakpoint recommended by CLSI, one isolate was categorized as not resistant with MIC value <2 µg/ml, one isolate was categorized as resistant with MIC value 4 µg/ml, three isolates were categorized as resistant with MIC value 16 µg/ ml, two isolates were categorized as resistant with MIC value 32 µg/ml, two isolates were categorized as resistant with MIC value 128 µg/ml, and three isolates were categorized as resistant with MIC value 256 µg/ml. By using the EtBr-agar Cartwheel method, results showed that isolates express efflux pump activity by different levels. The ATCC strain was found to fluoresce at EtBr concentration of 0.5 mg/l (negative control). The minimum concentration at which the adapted isolates showed fluorescence was 1 mg/l for isolates P1, P2, P3, and P4, while it was 1.5 mg/l for isolate P10, but isolate P8 was 2.5 mg/l, and isolates P11 and P12 were 3 mg/l; however, the isolates P6, P7 and P9 were more than 3 mg/l. The previous results revealed that isolates P6, P7, and P9 have a high efflux pump activity. Seven isolates (58.3%) were defined as imipenem resistance using the cut-off value of 1.5 cm according to CLSI recommendation using disk diffusion method. By using imipenem/EDTA combined disk test, the increase in inhibition zone with the imipenem/EDTA disk 7 mm or more than the imipenem disk alone was observed in five isolates [positive metallo-β-lactamase (MBL)] (Table 4).

All CIP-resistant isolates (n = 11) were subjected to genotypic detection for efflux pump genes presented in Table 1. Both MexR and MexZ genes were detected in all isolates (n = 11). NfxB gene and MexT gene were found in 3 isolates and 5 isolates, respectively. All genes were found collectively in three isolates (P3, P4, and P11). Concerning carbapenem genes, seven isolates (Imipenem-resistant) including five isolates (positive MBL) that previously showed positive results on phenotypic screening and

Table 2. Resistance percentages of *P. aeruginosa* toward different antibiotics.

Antibiotic family	Antibiotic	Resistance pattern (n = 12)			
		Resistant		Nonresistant	
		No.	%	No.	%
Carbapenems	MEM	12	100	0	0
	IPM	7	58.3	5	41.7
Aminoglycosides	AK	8	66.7	4	33.3
	CN	11	91.7	1	8.3
Fluoroquinolones	CIP	11	91.7	1	8.3
Cephems	CTX	12	100	0	0
	FOX	12	100	0	0
	CRO	12	100	0	0
	FEP	9	75	3	25
B-lactam combination	CTC	12	100	0	0

Table 1. Primer sequences used in this study.

Resistance mechanism		Gene	Product size (bp)	Sequence	Reference
Efflux pump activity	MexAB-OprM	MexR	637	F: CGCCATGGCCCATATTGAG R: GGCATTCGCCAGTAAGCGG	Osman *et al.*, 2018
	MexCD-OprJ	NfxB	939	F: CG ATCCTTCCTATTGCACG R: GCCAAGTGCCAGTATCG	
	MexEF-OprN	MexT	997	F: CGGTTGCAGCCTCTAGCC R: CGATTTTCCCGTTGCGACG	
	MexXY-OprM	MexZ	781	F: AGCGGCGCGACAGTAGCATA R: CCGAGGACCAGCGCAGGC	
Carbapenems	MBLs	*bla*IMP	232	F:GGAATAGAGTGGCTTAAYTCTC R: GGTTTAAYAAAACAACCACC	Alkhudhairy and Al-Shammari, 2020
	MBLs	*bla*VIM	390	F: GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG	

two isolates (negative MBL) were subjected to *bla*VIM and *bla*IMP genes detection. Results showed that blaVIM gene was found only in one isolate (Table 4).

DISCUSSION

Pseudomonas aeruginosa is commonly known to cause healthcare-acquired infections showing high rates of mortality. Recently, many studies had been conducted to study bacterial infections among children, especially *P. aeruginosa* (Bitsori *et al*., 2012; Hassuna *et al*., 2015; Logan *et al*., 2017). We found that the frequency of *P. aeruginosa* in children infections reported differs according to the region. In Omani's study published in 2015, among children uropathogens, the low frequency of *P. aeruginosa* was reported (2.86%) (Sharef *et al*., 2015). Similar low frequencies of 2.3% and 5.9% were also reported in Saudi Arabia among children with urinary tract infection (Alshamsam *et al*., 2009; Hameed *et al*., 2019) and 11.54% among children diagnosed with respiratory tract infections (Walid *et al*., 2016). In the United Arab Emirates, *P. aeruginosa* (36%) was the most common bacteria in CF children (Redha and Panickar, 2016). In 2018, a study conducted on children patients revealed that *P. aeruginosa* was the most prevalent by frequently 16.67%; those bacteria were found to be MDR. The authors suggest that this may be due to the high prevalence of the use of invasive devices during the period of hospitalization (El-Nawawy *et al*., 2018).

Table 3. Source and virulence factors distribution among *P. aeruginosa* strains.

Isolates	Source	Virulence factors		
		Biofilm interpretation	Pyocyanin production	
			Visual detection	Concentration (µg/ml)
P1	ETA	++	–	–
P2	ETA	++	–	–
P3	ETA	++	–	–
P4	ETA	–	+	1.23
P5	Urine	+	+	1.10
P6	Urine	–	–	–
P7	ETA	+	–	–
P8	ETA	+	–	–
P9	Sputum	–	–	–
P10	Sputum	–	+	0.836
P11	Sputum	+	–	–
P12	Sputum	+	–	–

For biofilm interpretation: - = nonbiofilm; + = weak biofilm; ++ = moderate biofilm; ETA = endotracheal aspirate. For pyocyanin production: -: negative and+: positive.

Table 4. MBL screening using imipenem/EDTA combined disk test (CDT) and the occurrence of *bla*VIM and *bla*IMP genes.

Isolates	MBL screening			Index	MBL genes
	Inhibition zones (cm)				
	EDTA	IPM	IPM/EDTA		
P1	0.9	–	3	3	–
P2	–	1.4	1.9	0.5	–[a]
P4	2	1.4	2.6	1.2	–
P8	1.7	1.3	1.9	0.6	–[a]
P9	0.8	–	2.2	2.2	+
P10	1	–	2.7	2.7	–
P12	0.9	–	2.3	2.3	–
Control	2	2.2	2.5	0.3	nd

nd = not determined.
[a]Negative control.

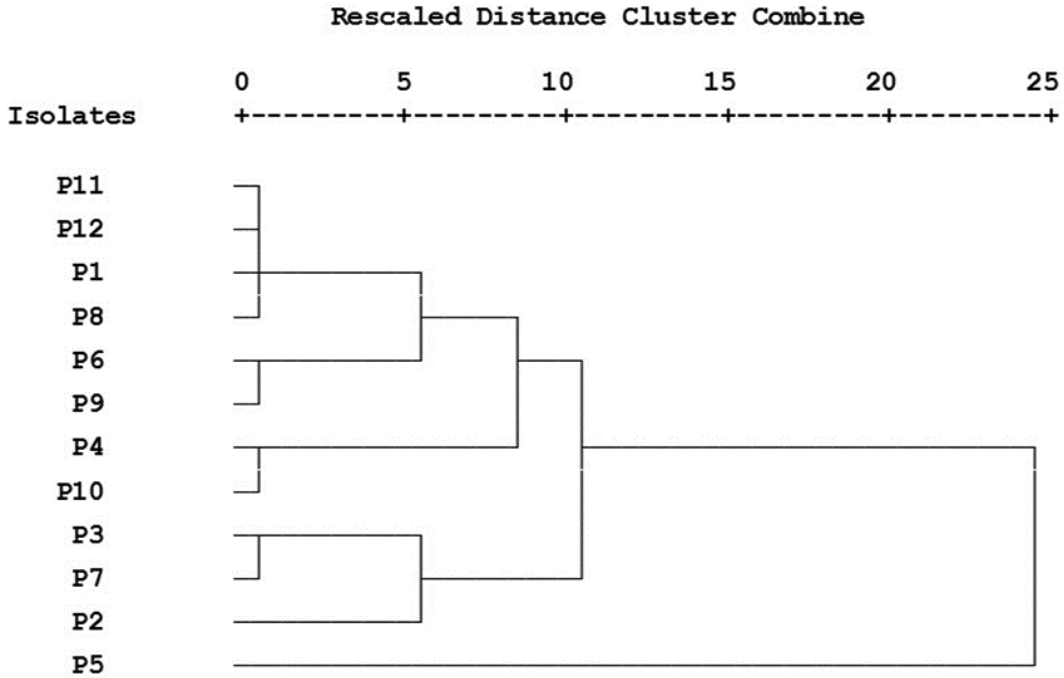

Figure 1. Hierarchical cluster analysis of different strains of *Pseudomonas aeruginosa.*

Besides the fact that *P. aeruginosa* is naturally resistant to many antimicrobial agents, they easily acquire resistance to new ones (Alnour and Ahmed-Abakur, 2017). This antimicrobial behavior significantly limits therapeutic options (Hirsch and Tam, 2010). Herein, high percentages of resistance to several antibiotics were detected (Table 2), at which a 100% resistance toward cephems antibiotics was observed; our results are in line with those reported about children (Hassuna *et al*., 2015; Pourakbari *et al*., 2016), but opposite to those reported among Spanish children (Ruiz-roldán *et al*., 2018) and Saudi children at which *P. aeruginosa* was found to be more sensitive to a range of antibiotics (Hameed *et al*., 2019).

Genetically, *P. aeruginosa* virulence is known to be multifactorial and combinatorial (Wendt *et al*., 2017). Such bacterial pathogens always share common mechanisms for their abilities to adhere, invade, survive host defenses, and cause infection (Wilson *et al*., 2002). The pathogenesis of *P. aeruginosa* infection is somehow related to its ability to synthesize some virulence factors (pyocyanin; proteases) and to form biofilms (Maisetta *et al*., 2020). In this study, biofilm formation ability was studied as one factor of the important virulence factors presented in *P. aeruginosa*, and as a result, 66.7% were able to form a biofilm at which their ability is ranging between moderate and weak. During our research journey, only a few types of research studied the biofilm formation among *P. aeruginosa* isolates from children's infections. Ralte *et al*. (2019) reported that, among different bacterial species isolated from children, the *P. aeruginosa* strain showed a strong ability to produce a biofilm. Biofilm formation *P. Aeruginosa* ability is an important process for bacterial colonization for persistence (Vallet *et al*., 2004), making it a problematic issue especially for patients requiring mechanical ventilation and catheterization (Alnour and Ahmed-Abakur, 2017). Inside those biofilms, bacteria became more resistant and difficult to eradicate than the planktonic ones (Mohamed *et al*., 2018b, 2020a); therefore, this mode of bacterial growth is strongly linked to various diseases at which many bacterial infections are biofilm-related (Mohamed *et al*., 2019, 2020c).

Three of our isolates are shown to produce pyocyanin pigment after 24 hours with different concentrations (Table 3). *P. aeruginosa* synthesizes a characteristic chloroform-soluble blue redox-active secondary metabolite called pyocyanin (Khadim and Marjani, 2019), which is considered an important virulence factor since it has a role to induce oxidative stress (Jazayeri *et al*., 2016). Cluster analysis was applied to detect the similarity of our *P. aeruginosa* strains phenotypic traits (Fig. 1).This analysis was performed to survey the prevalence of bacterial antibiotic susceptibility and complete phenotypic classification (Berrazeg *et al*., 2013).

It is reported that rates of antibiotic resistance among children *P. aeruginosa* infection are rising nationally (Logan *et al*., 2017). This bacteria can acquire new resistance mechanisms (Ruiz-roldán *et al*., 2018); the main ones for *P. aeruginosa* were mutation in target sites, changes in membrane permeability, the transmission of plasmid resistance genes, and efflux mechanism (Bejestani *et al*., 2015). In our study, two resistance mechanisms (efflux pump and MBL) were studied phenotypically and some genes have been detected genotypically. All CIP-resistant isolates showed higher MIC for EtBr than the standard strain using the phenotypic method. Reports of efflux pump percentages among children's *P. aeruginosa* infections were also found. Among children's *P. Aeruginosa* studied by Pourakbari *et al*. (2016), 62% showed an increased expression level of efflux pump genes. Helmy and Kashef (2017) reported that 23.5% of *P. aeruginosa* isolates showed multidrug efflux-mediated resistance. Many phenotypic methods have been used nowadays to detect MBL producing bacteria, which are majorly based on the ability to inhibit MBLs usingmetal chelators (Sachdeva *et al*., 2017). Our data identified that 41.67% of the *P. aeruginosa* isolates are MBL-positive using the combined imipenem/EDTA disk test method. In the United States, 11.3% of *Pseudomonas* was found to be carbapenem-resistant (Logan *et al*., 2017). Bejestani *et al*. (2015) reported that the frequency of imp gene was detected in 3.3% *P. aeruginosa* isolates; on the other hand, the vim gene was not detected in any of MBL-positive isolates (0%), at which this low frequency comes in line with our results.

CONCLUSION

This study highlights the high frequency of antibiotic resistance, besides the production of important virulence factors (biofilm formation and pyocyanin production). Also, the gene occurrence of antibiotic resistance mechanisms was reported among our *P. aeruginosa* from children. This virulent bacterial behavior is alarming which needs attention to the way we use antibiotics. However, studies with a larger number of *P. aeruginosa* children isolates are recommended.

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ETHICAL APPROVAL

This study involved microbes isolated in microbiology laboratory as a part of continuous routine laboratory work and was determined not to be human subject's research. The study did not involve clinical data for patients and did not involve laboratory animals or invasive procedures.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

Al Rashed N, Joji RM, Saeed NK, Bindayna KM. Detection of overexpression of efflux pump expression in fluoroquinolone-resistant *Pseudomonasaeruginosa* isolates. Int J Appl Basic Med Res, 2020; 10:37–42.

Alkhudhairy MK, Al-Shammari MMM. Prevalence of metallo-β-lactamase-producing *Pseudomonas aeruginosa* isolated from diabetic foot infections in Iraq. New Microbes New Infect, 2020; 35:100661.

Alnour TM, Ahmed-Abakur EH. Multidrug resistant *Pseudomonas* (P) *aeruginosa*: medical impact, pathogenicity, resistance mechanisms and epidemiology. JSM Microbiol, 2017; 5:1046.

Alshamsam L, Al Harbi A, Fakeeh K, Al Banyan E. The value of renal ultrasound in children with a first episode of urinary tract infection. Ann Saudi Med, 2009; 29:46–9.

Bejestani FB, Hakemi-vala M, Momtaheni R, Bagheri Bejestani O, Gholami M. The frequency of imp and vim Genes among *Pseudomonas aeruginosa* isolates from children ' s medical center of Tehran. Arch Clin Infect Dis, 2015; 10:e20991.

Berrazeg M, Drissi M, Medjahed L, Rolain JM. Hierarchical clustering as a rapid tool for surveillance of emerging antibiotic- resistance phenotypes in *Klebsiella pneumoniae* strains. J Med Microbiol, 2013; 62:864–74.

Bitsori M, Maraki S, Koukouraki S, Galanakis E. *Pseudomonas Aeruginosa* urinary tract infection in children: risk factors and outcomes. J Urol, 2012; 187:260–4.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 29th edition. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

El-Nawawy A, Ashraf GA, Antonios MAM, Meheissen MA, El-Alfy MMR. Incidence of multidrug-resistant organism among children admitted to pediatric intensive care unit in a developing country. Microb DRUG Resist, 2018; 24:1198–206.

Feghali PAR El, Nawas T. Extraction and purification of pyocyanin: a simpler and more reliable method. MOJ Toxicol, 2018; 4:417–22.

Hameed T, Al Nafeesah A, Chishti S, Al Shaalan M, Al Fakeeh K. Community-acquired urinary tract infections in children: resistance patterns of uropathogens in a tertiary care center in Saudi Arabia. Int J Pediatr Adolesc Med, 2019; 6:51–4.

Hassuna NA, Mohamed AHI, Eleuoon SMA, Rizk HAA. High prevalence of multi- drug resistant *Pseudomonas aeruginosa* recovered from infected burn wounds in children. Arch Clin Microbiol, 2015; 6:1–7.

Helmy OM, Kashef MT. Different phenotypic and molecular mechanisms associated with multidrug resistance in gram-negative clinical isolates from Egypt. Infect Drug Resist, 2017; 10:479–98.

Hirsch EB, Tam VH. Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res, 2010; 10:441–51.

Jazayeri JA, Nguyen K, Kotsanas D,Schneiders F, Tan C, Jazayeri M, Armstrong D. Comparison of virulence factors in *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients. J Med Microbiol Diagnosis, 2016; 5:1000242.

Khadim MM, Marjani MFAL. Pyocyanin and biofilm formation in *Pseudomonas aeruginosa* isolated from burn infections in Baghdad, Iraq. Jordan J Biol Sci, 2019; 12:31–5.

Lima JLDC, Alves LR, Jacomé PRLA, Bezerra Neto JP, Maciel MAV, Morais MMC. Biofilm production by clinical isolates of *Pseudomonasaeruginosa* and structural changes in LasR protein of isolates non biofilm-producing. Braz J Infect Dis, 2018; 22:129–36.

Logan LK, Gandra S, Mandal S, Klein EY, Levinson J, Weinstein RA, Laxminarayan R. Multidrug- and carbapenem-resistant *Pseudomonasaeruginosa* in children, United States, 1999–2012. J Pediatric Infect Dis Soc, 2017; 6:352–9.

Maisetta G, Grassi L, Esin S, Kaya E, Morelli A, Puppi D, Piras M, Chiellini F, Pifferi M, Batoni G. Targeting *Pseudomonasaeruginosa* in the sputum of primary ciliary dyskinesia patients with a combinatorial strategy having antibacterial and anti-virulence potential. Int J Mol Sci, 2020; 21:69.

Mohamed MSM, Mostafa HM, Mohamed SH, Abd El-Moez SI, Kamel Z. Combination of silver nanoparticles and vancomycin to overcome antibiotic resistance in planktonic/biofilm cell from clinical and animal source. Microb DRUG Resist, 2020a; http://doi.org/10.1089/mdr.2020.0089.

Mohamed SH, Elshahed MMS, Saied YM. Evaluation of Honey as an antibacterial agent against drug-resistant uropathogenic *E.coli* strains. Res J Pharm Technol, 2020c; 13(8):3720–4.

Mohamed SH, Khalil MS. DNA sequencing of bla SHV genes among uropathogenic *Klebsiellapneumoniae* harboring bla CTX-M-15. Int J Pharm Res, 2020; 12:443–9.

Mohamed SH, Khalil MS, Azmy M. *In vitro* efficiency of ampicillin, thymol and their combinations against virulence strains of *Klebsiellapneumoniae*. Int J Pharm Res, 2019; 11:315–21.

Mohamed SH, Khalil MS, Mohamed MSM, Mabrouk MI. Prevalence of antibiotic resistance and biofilm formation in *Klebsiellapneumoniae* carrying fimbrial genes in Egypt. Res J Pharm Technol, 2020b; 13(7):3051–8.

Mohamed SH, Mohamed MSM, Khalil MS, Azmy M, Mabrouk MI. Combination of essential oil and ciprofloxacin to inhibit/eradicate biofilms in multidrug-resistant *Klebsiellapneumoniae*. J Appl Microbiol, 2018b; 125:84–95.

Mohamed SH, Mohamed MSM, Khalil MS,Mohamed WS, Mabrouk MI. Antibiofilm activity of papain enzyme against pathogenic *Klebsiellapneumoniae*. J Appl Pharm Sci, 2018a; 8:163–8.

Mohamed SH, Salem D, Azmy M, Fam NS. Antibacterial and antibiofilm activity of cinnamaldehyde against carbapenem-resistant *Acinetobacterbaumannii* in Egypt: *in vitro* study. J Appl Pharm Sci, 2018c; 8:151–6.

Morales E, Cots F, Sala M, Comas M, Belvis F, Riu M, Salvadó M, Grau S, Horcajada JP, Montero MM, Castells X. Hospital costs of nosocomial multi-drug resistant *Pseudomonasaeruginosa* acquisition. BMC Health Serv Res, 2012; 12:122.

Osman ME, El-hendawy HH, El-all SMA, Hassan AM, Mahmoud DE. Correlation between resistance of *Pseudomonasaeruginosa* to benzalkonium chloride and expression of efflux pump genes. J Appl Pharm Sci, 2018; 8:44–52.

Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance *Pseudomonasaeruginosa* in intensive care unit; a critical review. Genes Dis, 2019; 6:109–19.

Pourakbari B, Yaslianifard S, Yaslianifard S, Mahmoudi S, Keshavarz-Valian S, Mamishi S. Evaluation of efflux pumps gene expression in resistant *Pseudomonasaeruginosa* isolates in an Iranian referral hospital. Iran J Microbiol, 2016; 8:249–56.

Ralte Z, Naina P, Amladi A, John M, Anndan S, Varghese AM. Determination of biofilm-forming capacity of otopathogens isolated from discharging ears in children with chronic otitis media. Indian J Med Microbiol, 2019; 37:442–5.

Redha KY Al, Panickar J. Bacteriology among CF children in United Arab Emirates. J Cyst Fibros, 2016; 15:S70.

Ruiz-roldán L, Bellés A, Bueno J, Azcona-Gutiérrez JM, Rojo-Bezares B, Torres C, Castillo F, Sáenz Y, Seral C. *Pseudomonasaeruginosa* isolates from Spanish children: occurrence in faecal samples, antimicrobial resistance, virulence, and molecular typing. Biomed Res Int, 2018; 2018:8060178.

Sachdeva R, Sharma B, Sharma R. Evaluation of different phenotypic tests for detection of metallo -β- lactamases in imipenem-resistant *Pseudomonasaeruginosa*. J Lab Physicians, 2017; 9:249–53.

Sharef SW, El-Naggari M, Al-Nabhani D, Al Sawai A, Al Muharrmi Z, Elnour I. Incidence of antibiotics resistance among uropathogens in Omani children presenting with a single episode of urinary tract infection. J Infect Public Health, 2015; 8:458–65.

Singh I, Jaryal SC, Thakur K, Sood A, Grover PS, Bareja R. Isolation and characterization of various *Pseudomonasspecies* from distinct clinical specimens. IOSR J Dent Med Sci, 2015; 14:80–4.

Vallet I, Diggle SP, Stacey RE, Cámara M, Ventre I, Lory S, Lazdunski A, Williams P, Filloux A. Biofilm formation in *Pseudomonasaeruginosa*: fimbrial cup gene clusters are controlled by the transcriptional regulator MvaT. J Bacteriol, 2004; 186:2880–90.

Walid J, Moshref L, Alzanbagi M, Moshref R, Baabdullah W, Aldigs E, AlAhmadi T, Kari J, Alharbi S, Safdar O, Felemban O. Antibiotic resistance pattern in healthy children diagnosed with community acquired respiratory tract infections in King Abdulaziz University Hospital. J Med Microbiol Diagn, 2016; 5:1000232.

Wendt M, Silva BCJ De, Heo G. Virulence factors and antimicrobial resistance of *Pseudomonasaeruginosa* isolated from Pet Turtles. Asian J Anim Vet Adv, 2017; 12:205–11.

Wilson JW, Schurr MJ, LeBlanc CL, Ramamurthy R, Buchanan KL, Nickerson CA. Mechanisms of bacterial pathogenicity. Postgrad Med J, 2002; 78:216–24.

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