Detection of Antiseptic Resistant Genes in Colistin- Resistant *Pseudomonas aeruginosa* and MDR *Klebsiella penumoniae*

Noor S. Hassan[1], Mohamed F. Al –marjani[2], Nadema H. Hussain[3]

[1]Researcher, [2]Professor, [3]Assistant Professor, Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq

Abstract

Objectives: The aim of this study was to detect the existence of developments in resistance to biocide of Pseudomonas *aeruginosa* resist of colistin and in multi-drug resistance *Klebsiella pneumonia* in the hospital environment. Materials and Methods: The study included 25 isolates of *K. pneumoniae* and 30 isolates of *P. aeruginosa*. Isolated from different clinical and environmental samples in Baghdad hospitals. Antibiotic sensitivity tests and their susceptibility to multiple antibiotic resistance and sensitivity tests were studied for the most commonly used antiseptics at the preventive level (benzalkonium chloride). The test was carried out using the micro dilution broth method, following Institution of Clinical and Laboratory Standards guidelines, PCR was performed for detection of bla_{TEM}, bla_{SHV} , bla_{CTX-M} , *qac*C/D, *qac*ΔE1and *qac*E beta lactamase and antiseptic genes. Results: A high rate of multiple resistance to the most used antibiotics was observed, so the rate of resistance to all antibiotics that was used was 16.0% of *P. aeruginosa* and 4% of *K. pneumoniae* possesses comprehensive resistance to all antibiotics that were used and the resiste of colistin in *P. aeruginosa* was 36% .The prevalence of ESBLs was 36.0% and 48.0 % of *P.aeruginosa* and *K.penomoniea* respectively ,in addition to their strong ability to form biofilms 80 % in *P.aeruginosa* and 94% in *k.pneumoniea* and their ability to resist Antiseptics . The percentage of resistance to antiseptic of benzilkonium chloride showed the highest concentration of *P.aeruginosa* was 33.3% and *K. pneumoniae* 37.5% . The result of ESBL and antiseptic genes detection clarify, the percent of production genes were (10%), (40%) blaTEM; (6.66%),(56%)bla_{SHV} ;(33%),(64%) bla_{CTX-M} ;(70%),(44%) *qac*C/D; (80%),(56%) *qac*ΔE1 in *P.aeruginosa* and *K.pneumoniea* respectively and no any isolate carried *qac*E gene. production of extended spectrum β-lactamase genes in addition to their strong ability to form biofilms 80 % in *p.aeroginosa* and 94% in *k.pneumoniea* and their ability to resist a antiseptics . The percentage of resistance to antiseptic of benzilkonium chloride showed the highest concentration of *P. aeruginosa* was 33.3% and *K. pneumoniae* 37.5%, the result of beta lactamase and antiseptic genes detection clarify PCR was performed for detection of bla_{TEM}, bla_{SHV} , bla_{CTX-M} , *qac*C/D, *qac*ΔE1and *qac*E beta lactamase and antiseptic genes . Conclusion: Our observations indicate that there is a significant correlation between the ability of bacteria to resist multiple antibiotics in addition to their ability to resist the most commonly used antiseptics, due to their physiological nature and increased virulence factors.

Keywords: *Antiseptic, Antibiotic Resistant genes, Pseudomonas aeruginosa, Klebsiella penumoniae*

Introduction

Hospital infection represents one of the most common challenges facing health systems in the developed world such as Health Care - Associated Infection (HCAI) as the number of hospitals which acquired infections (HAI) is increasing dramatically worldwide, especially due to the emergence of multidrug-resistant bacteria (MDR) [1]. MDR isolates spread is easily observed in hospitals settings, and are seen specially in a patient while under medical care in a hospital or other healthcare facility. This infection can occur during health care provision for other illnesses and even after patients have discharged the disease, an occupational infection may form among medical personnel [2]. There are two types of bacteria that are common in nosocomial and respiratory infections, *Klebsiella penumoniea* and *Pseudomonas aeruginosa* that most often cause human nosocomial infections

[3]. *K. penumoniea* and *P. aeruginosa* have the ability to form biofilm on medical devices, such as catheters and ventilators. In some cases, pneumonia is excessive and very violent and can spread and affect healthy people [4], causing life-threatening and often community-transmitted infections, along with pyogenic liver abscess, meningitis, necrotizing fasciitis, endometriosis, and acute pneumonia [5].

Colistin is a perfect antibiotic against Gram-negative bacteria, the most important of which is *Pseudomonas aeruginosa* [6]. Where it is effective against the outer membrane of bacteria that are negative for the Gram stain, specifically anionic lipopolysaccharide (LPS) [7]. Of concern is the high rate of resistance to *pseudomonas aeruginosa* to colistin [8], as this is explained by the bacteria's possession of two main mechanisms of resistance to colistin in Gram-negative bacteria, mutation and adaptation, as the resistance resulting from mutations is the increase in gene expression on the reflux pumps, Adaptive is the ionic change of the ions components present in the cell membrane [9].

Antiseptics and disinfectants are widely used in hospitals and other healthcare settings to prevent infection and reduce the chances of contracting diseases and epidemics [10], despite the widespread use of disinfectants, a high rate of microbial contamination has recently been observed. This may be due to the development of multiple virulence factors and resistance to both antibiotics and disinfectants due to its ability to adapt to the indiscriminate use of near-lethal concentrations of disinfectants [11].

K. pneumoniae and *P. aeruginosa* are dangerous micro-organisms, capable of growing on solid, non-porous surfaces, in addition to their possession of multi virulence factors, the most important of which is their ability to form biofilms There is a wide range of active chemical agents (or "biocides") in these products, and many have been used for hundreds of years for sterilization, disinfection and preservation [12].

Biocides have a wider range of effect compared to antibiotics, as antibiotics have specific intracellular targets in addition to their specialization, while biocides have comprehensive and non-specific effectiveness. Nevertheless, the widespread use of disinfectants has led to pathogens acquiring resistance factors that may be common with antibiotic resistance and what is known as cross resistance [13]. It is important to note that many of these biocides can be used alone or with a variety of products, which differ widely in their activity against microorganisms [12]. Antiseptics differ according to their effectiveness towards the vital cell. Some of them target cell membranes, plasma membranes, nucleic acids, or they may be oxidizing agents. Therefore, hospital disinfection has a major role to play in controlling health care-related [14]. Disinfectants play an essential role in controlling infection and preventing the transmission of infectious pathogens in hospitals [15]. The aim of the study is to detection the antiseptic resistance genes and the extent of their prevalence in multidrug resistant clinical and environmental bacterial isolates in hospital.

Materials and Methods

Samples Collection:

Fifty-five samples were collected from clinical and environmental sources, 30 isolates of *Pseudomonas aeruginosa* and 25 isolates of *Klebsiella pneumoniae*, initially diagnosed in hospitals. The samples included swabs for burns, wounds, urine, sputum, and swabs from intensive care rooms, operating rooms, main operating rooms, patient halls, and the children's protection hall, in addition to swabs for fluid withdrawal devices, endoscopes, surfaces and sinks for preterm infants, which were collected during the period from July to October 2019.

Bacterial Isolates:

Primary diagnosis based on morphological characteristic of the colonies that included colony shape, texture, color and edges dependently on bacterial growth on the MacConky agar and blood agar. All isolates were identified using conventional biochemical tests and vitek 2 system.

Antibiotic Susceptibility Test:

The susceptibility of isolates to different antibiotics was tested using the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute guidelines [16]. Using antibacterial agents included: gentamicin (GM), amoxi clav (AUG), amikacin (AK), ceftriaxone (CRO), levofloxacin (LEV), deoxycycline (DXC), piperacillin tazobactam (PTZ), ceftazidime

(CAZ), cefazolin (KZ), aztreonam (ATM), tetracycline (T), cefepim (EFEP) and colistin (CO). The bacterial culture was carried out using Muller-Hinton agar medium (Himedia, India) and the bacterial suspension prepared with a dilution standard corresponding to the McFarland standard, after which the cultivated plates were incubated at 35 ° C for eighteen hours.

Detection ESβLs by using Vitek-2 system :

Detection Phenotypic of ESβLs producing isolates were also done by Vitek-2 system by using sensitivity of antibiotic test Number (AST-GN69) card according to the manufacturer's instructions.

Detection of Biofilm formation by Micro titer plate assays:

In this study, isolates of *P. aeruginosa* and *K. pneumoniae* were examined for their ability to form biofilms. A micro titer plate was used according to the method described by [17] , [18]. Twenty µl of the bacterial suspension was taken from an overnight culture to inoculate the micro titer wells containing 180 µl of Brain Heart Infusion (BHI) broth with 1% sucrose. Thus, the control wells which contained 200 µl of BHI broth with the bacterial suspension. Then the micro titer plate was closed and covered with Para film during incubation at 37 ° C for twenty-four hours. Unlinked bacterial cells were surely removed by washing the wells three times with PBS (pH 7.2), then kept at room temperature for fifteen minutes in order to dry, then 200 µl of crystal violet (0.1%) was added to the wells for a period of time. Fifteen minutes. After removing the crystal violet solution, the wells were washed three times with PBS (pH 7.2) to remove the unbounded dye and allowed to dry at room temperature, after which 200 µl of 95% ethanol was used for the purpose of extraction. And in Final, the optical density value of each well is deducted by an ELISA reader at 630 nm absorption degree.

Determination of Minimal Inhibitory Concentration (MIC) for chemical antiseptics:

Fill all wells in a 100 µl micro titer plate of BHIB , after that 100 µl of activated isolates suspension after adjusting turbidity to 1 x 10^8 cfu / ml as 0.5 McFarland with normal saline is added to the wells in a micro titer plate. and then the detergent concentration is then diluted using the two-prong dilution method until we obtain a series of dilutions with pre-added BHIB from high to low concentration distributed in wells (A to H) in wells (1-10). The row of wells A12- H12 is considered as a positive control. Whereas (A11 - H11) which is a passive control. After incubation for 18 hours at 37 ° C, add 60 µl of resazurin sodium to each well in the dish and leave it for (2-4) hours in the incubation to observe the color change [19].

Molecular detection of β-lactamase and antiseptic genes using PCR technique:

All isolates were submitted to PCR technique to detection for ESBLs and antiseptic genes; bla_{TEM}, bla_{SHV} , bla_{CTX-M} , *qac*C/D, *qac*ΔE1and *qac*E by using Specific primers (table 1) [20]. DNA of isolates were extracted by using a commercial purification system (Genomic DNA Purification Kit) and PCR was used to amplify genes. PCR mixture was set up for each gene alone in a total volume of 25 µl included 12.5µl of Go Taq Green Master Mix, 1.5 µl of each primer (10 picomole/ µl) and 4 µl of template DNA. The volume remaining was completed with sterile nuclease free water PCR products were detected by agarose gel electrophoresis. A DNA marker (Promega/USA) was run with each gel, and the genotype was determined by the size of the amplified produce

Table 1: The sequences of ESβL and antiseptic primers used in this study

Primer	Sequences (3'-----5')	Product size (bp)	Reference
qacC/D	F:GGCTTTTCAAAATTTATACCATCCT R: ATGCGATGTTCCGAAAATGT	249	Sidhu et al., (2002)
qacΔE1	F: AATCCATCCCTGTCGGTGTT R:CGCAGCGACTTCCACGATGGGGAT	155	Zou et al., (2014)
qacE	F: AAGTAATCGCAACATCCG R: CTACTACACCACTAACTATGAG	246	Zou et al., (2014)
blaTEM	F-ATGAGTATTCAACATTTCCGTG R-TTACCAATGCTTAATCAGTGAG	861	Szabo et al., (2005)
blaSHV	F-ATTTGTCGCTTCTTTACTCGC R-TTTATGGCGTTACCTTTGACC	1051	Szabo et al., (2005)
blaCTX-M	F- CGCTTTGCGATGTGCAG R- ACCGCGATATCGTTGGT	544	Szabo et al., (2005)

Results and Discussion

In this study 55 samples were collected from clinical and environmental sources. All samples were transferred to the laboratory by transport media, then cultured on MacConkey agar and blood agar and incubated for 18-24 h at 37 ° C. Then the isolates were diagnosed and confirmed by using a VITEK-2 pressurized system according to the manufacturer's instructions (Biomerieux / France). The study included resistance of these isolates to antibiotics and antiseptics in addition to their possession of virulence factors and their ability to form biofilms [21]. *Klebsiella pneumoniea* is a type of Gram-negative bacteria and can cause different types of health care-related infections. *Klebsiella* infection usually occurs among patients who are receiving treatment for other conditions. Patients whose care requires devices such as ventilators (ventilators) or intravenous tubes for drugs and urinary catheters [22]. Patients who take long courses of some antibiotics are more likely to develop a *Klebsiella* infection [23]. Antibiotic susceptibility testing was performed *P. aeruginosa* and *K. pneumoniae isolates*, results showed that isolates (36%) of P. *aeruginosa* were resistant to colistin, all isolates were resistant to amoxiclav, tetracycline and doxycycline (100% each). In addition, 46% of the P. *aeruginosa* isolates were resistant to gentamicin and to tazobactam. In K. *pneumoniea*, the percentage of resistance to gentamycin was 20% and to tazobactam 28%. cefazolin and aztreonam 90% of *P.aeruginosa* and in *K.pneumoniea* rate of resist to aztreonam was 64% and in Cefazolin was 72%and the same percentage that was in the case of ceftazidime. And about the resistance rate in the pseudomonas, it was 50%, for amikacin resistance 40% of *P.aeruginosa* isolates and 16% for *K.pneumoniea* isolates, and for levofloxacin resistance was 53% for the *P.aeruginosa* isolates and 36 % for the *K.pneumoniea* isolates, and the cefepime resistance was 46% in all isolates. *K. pneumoniae* showed greater resistance to ciprofloxacin 63% in *P.aeruginosa* and 68% in *K. pneumoniea* as shown in the table 2 .

During the past decade, observe increasing in rates of antimicrobial resistance has been recognized worldwide,

and an increased frequency of MDR isolates has also been demonstrated in clinical environment samples. Where the term drug resistance was applied to isolates for which no treatment options were available [24].

It has been observed during this study that the bacteria communities follow different strategies in antibiotic resistance, which may be physiological or genetic. In terms of clinical isolates, they gain resistance to multiple antibiotics due to patients being exposed to long periods of treatment with these antibiotics, in addition to the irregular use and irregular concentrations of these antibiotics, which led to a gradual adaptation of pathogens to generate defenses against them and one of the most important of these methods is refluxes pumps one of the most important means of multiple antibiotic resistance [25], While isolates taken from hospital environment and medical devices that indicate contamination of the hospital environment and medical devices with microbes have multiple resistance to antibiotics as their resistance to antibiotics varies according to the source of contamination [26]. If the source of contamination is from the soil, which is the home of bacterial isolates where they acquire self-resistance to antibiotics produced by other bacterial types present in the soil, and the other source is medical waste, which is similar to clinical samples that come from infected patients [25].

In this study, the bacterial isolates showed their ability to produce extended spectrum beta lactamase included (36.0%) of *P.aeruginosa* isolates and (48.0%) of *K.pneumoniae* isolates were ESBLs produced. This is in line with the study where included (42.30%) of *P. aeruginosa* isolates were positive for ESβLs, and in another study by [27] showed that (46.2%) of *K.pneumoniae* isolates were positive for ESβLs. This is consistent with what we have reached, and in a comprehensive study made by [28] it was shown that (50%) of *Klebsiella sp* were positive for ESβLs.

P. aeruginosa and *K. pneumoniae* were from clinical and environmental sources as MDR and have ability to biofilm form biofilm was assessed by a micro titer plate. The results showed that 80% of *P. aeruginosa* and 96% of *K. pneumoniae* were production biofilms, The results were consistent with the results of other studies, in study by [29] results showed (85.63%) of *P. aeruginosa* producing biofilms. We have shown the similar study reported by Hassan and other [30,31] *K. pneumoniae* (64.7%) as high or medium productive biofilms and 40 isolates (35.3%) were identified as poorly produced biofilms. Saifi *et al.* [32] Reported that the majority of *K.pneumoniae* (93.6%) were biofilms and only 6.4% were not biofilms , The percentage observed of biofilm formation in clinical samples increased significantly compared to environmental samples. These differences in levels of formation biofilm in clinical samples may be the result of repeated use of drugs, also with high period duration of antibiotic treatment, and excessive use of drugs in the animal and poultry industry that is consumed by humans.

It is worth mentioning that the incorrect use of disinfectants and detergents and the increase of mutagenic bacteria in today's industrial life. As isolation of clinical source has some selectivity in the formation of biofilms, which is related to the presence of proteins necessary for the growth and biofilms formation associated with the outer membrane, and we note that these membrane proteins need time to adapt to the conditions. It can be seen that the surrounding environment conditions provided in the clinical samples are suitable for them and do not need to be adapted.

Table 2: Antibiotic Susceptibility of *P.aeruginosa* and *k.penumoniea*

NO.	Antibiotic	Percentage of resistance (%) in P.aeruginosa isolates	Percentage of resistance (%) in k.penumoniea isolates
1	Amoxi-clav 30µg	100	100
2	Ceftazidim 30 µg	50	72
3	Tazobactam 30 µg	46	28
4	Ceftriaxone 10 µg	63	68
5	Deoxycycline 10 µg	100	100
6	Amikacin 10 µg	40	16
7	Cefepim 10 µg	46	46
8	Tetracyclin 30 µg	100	100
9	Aztreonam 30 µg	90	64
10	Levofloxacin 10 µg	53	36
11	Cefazolin 30 µg	90	72
12	Gentamycin 10 µg	64	20
13	Colistin 10 µg	36	0

Antibiotic resistance and biofilm formation among hospital pathogens is a risk that is difficult to eliminate over time. Producing lactamase and spread among bacterial pathogens adversely affects the possibilities of antibiotic therapy, which is worth noting biocides play an important role in preventing and controlling hospital-acquired infections. The lowest antimicrobial agent for the minimum inhibitor concentration (MIC) is usually tested, and the purpose of this test is to study and find out the sensitivity of bacteria to the antiseptic and to know the lowest concentration that may affect them [33]. The results of the study showed the minimum inhibitory concentration (MICs) for (Benzalkonium chlorid), were 33. 3% of *P. aeruginosa* and 37.5% of *K. pneumoniae* isolates had MIC 40000 µg/ml (4%) , whereas 62.5% of *P.aeruginosa* and 50% *K. pneumoniae* isolates had MIC 20000 µg/ml (2%), while 66.6% of *P.aeruginosa* and 62.5 % *K. pneumonia* isolates had MIC 10000 µg/ml (1%),fig.1

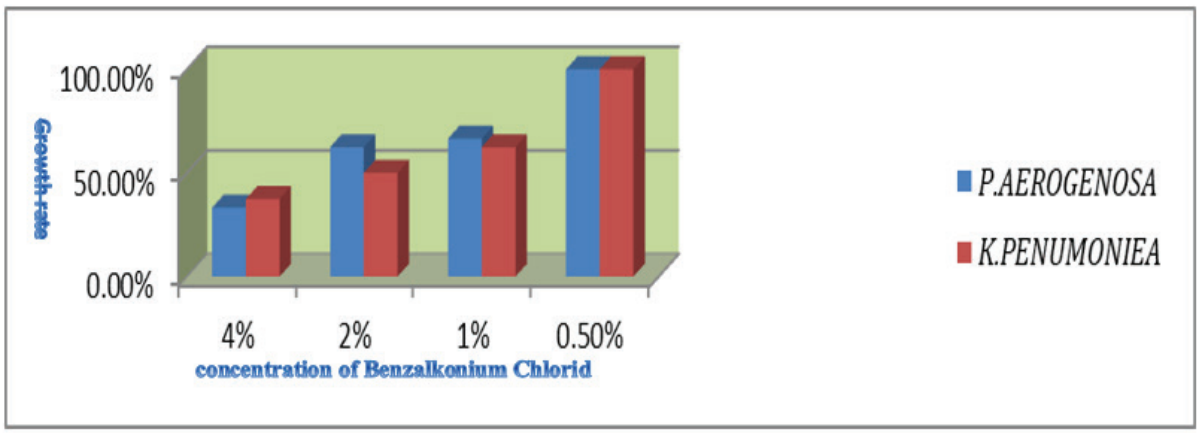

Figure (1): Dilution to concentration of Benzalkonium Chlorid

The emergence of this resistance is related to the ability of bacteria to adapt to these antiseptics through the acquisition of virulence factors represented in the formation of the cell wall and reflex systems, in addition to their acquisition of antiseptic resistance genes, which may have close relationship with the antibiotic resistance genes, and this resistance has increased specifically in the recent times. by using disinfectants with near-fatal concentrations of the microbe, it gave the opportunity to coexist and develop for resistance and survival [34].

By using PCR The results of detection of extended spectrum β- lactamase and antiseptic genes showed the presence of *qac*ΔE shows in (80%) of *P.aeruginosa* and in (56.0%) of *K.pneumoniae* isolates; *qac* C/D shows in (70.0%) of *P.aeruginosa* and in (44.0%) of *K.pneumoniae*; bla $_{CTX}$ in (33.0%) of *P.aeruginosa* and in (64.0%) of *K.pneumoniae* isolates; bla $_{SHV}$ in (6.66%) of *P.aeruginosa* and in (56.0%) of *K.pneumoniae* ; bla $_{TEM}$ shows in (10.0%) of *P. aeruginosa* and in (40.0 %) of *K.pneumoniae* and no any isolate carried *qac* E genes , as shown in the table 3.

Table 3: Percentage of ESβLs and *qac* genes in Bacterial species

Bacterial species	No. Clinical isolate	No. Environmental isolate	Genes %
P.aeruginosa	16	11	qacΔ E 80
	13	8	qac C/D 70
	5	5	bla CTX 33
	0	3	bla TEM 10
	1	1	bla SHV 6
K.pneumoniae	14	2	qacΔ E 56
	10	1	qac C/d 44
	16	1	bla CTX 64
	9	1	bla TEM 40
	14	0	bla SHV 56

In a study conducted in Iraq by [35] revealed that all *P. aeruginosa* isolates (100%) carry bla $_{CTX}$, Bokaeian *et al* [36] stated that the percentage of bla $_{TEM}$ gene was (100%) in *P.aerugenosa* Iran . Mohamed *et al* [37] reported that (90%) of *K.pneumoniae* isolates carried bla $_{TEM}$ and bla $_{CTX}$ genes respectively in Egypt, and another study was conducted in Egypt, and [38] reported that the percentage of bla $_{CTX-M}$ is (53.3%) in *K. pneumoniae* isolates in a The study by [39] found that the percentage of the bla $_{TEM}$ gene is 71.7% and the bla $_{CTX-M}$ gene is 99.2% in *K. pneumoniae* isolates. Excessive, semi-fatal and intense use led to the emergence of bacterial isolates that are clearly resistant to the dangerous concentrations of disinfectants that are not only resistant to antibiotics. Special genes responsible for host activities such as multiple flow systems and the possibility for these genes to be transmitted between bacterial strains through direct contact or via mobile plasmids [40].

Hilal *et al*. [41] revealed that *qac*EΔ1 and *qac*E gene replication and their association with antibiotic and biocide resistance in *Pseudomonas aeruginosa* isolates in Egypt. It was found that the percentage of *qac*E1 gene was 57.8% in MDR isolates and 13.4% in bacterial isolates. Sensitive to several drugs, while the secreted mucilage was only present among the multidrug- resistant *P. aerugenosa,* while conducted by Amazonian 2014 in a study Maliocytes by [42], the percentage of quaternary ammonium complex resistance genes (QACs) in *P. aeruginosa* was (14.28%) Contained the *qac*C / D gene while the *qac*EΔ1 gene was 100%

In another study by [43], the *qac*EΔ1 gene was detected in 48% of an isolate. Eighty-eight percent of the poly-resistant isolates carried the *qac*EΔ1 gene, while 35% of the non-resistant isolates were positive for this gene, and multiple resistance was well correlated with its presence.

Among the isolates tested, more than one study has been reported on *K. pneumoniae* by [44]; Abu Zayd and Ames in the UK showed that susceptibility to disinfectants was decreased due to the presence of the *qac*ΔE1 and *cep*A genes.

Conclusion

Our observations indicate a significant correlation between the ability of bacteria to resist many antibiotics in addition to their ability to resist the most common antiseptics due to their physiological nature and increased virulence factors, noting the high incidence of *pseudomonas aeruginosa* against colistin and the emergence of multiple antibiotic resistance isolates of *Pseudomonas aeruginosa* and *Klebsiella Pneumonia* and high incidence of genes responsible for beta-lactam resistance and antiseptic resistance .

Funding: Self-Funding.

Author Conflict: Nil

Ethical Clearance: Taken from education committee of biology department, college of science, Mustansiriyah University, Baghdad, Iraq.

References

1. Haque M, Sartelli M, McKimm J, Bakar MA. Health care-associated infections – An overview. Vol. 11, Infection and Drug Resistance. 2018. p. 2321–33.
2. Magiorakos AP, Burns K, Rodríguez Baño J, Borg M, Daikos G, Dumpis U, et al. Infection prevention and control measures and tools for the prevention of entry of carbapenem-resistant Enterobacteriaceae into healthcare settings: Guidance from the European Centre for Disease Prevention and Control. Antimicrobial Resistance and Infection Control. 2017;6(1).
3. El Chakhtoura NG, Saade E, Iovleva A, Yasmin M, Wilson B, Perez F, et al. Therapies for multidrug resistant and extensively drug-resistant non-fermenting gram-negative bacteria causing nosocomial infections: a perilous journey toward 'molecularly targeted' therapy. Expert Review of Anti-Infective Therapy. 2018;16(2):89–110.
4. Guo M, Feng C, Ren J, Zhuang X, Zhang Y, Zhu Y, et al. A novel antimicrobial endolysin, LysPA26, against Pseudomonas aeruginosa. Frontiers in Microbiology. 2017;8(FEB).
5. Keynan Y, Rubinstein E. The changing face of Klebsiella pneumoniae infections in the community. Vol. 30, International Journal of Antimicrobial Agents. 2007. p. 385–9.
6. Dosler S, Karaaslan E, Alev Gerceker A. Antibacterial and anti-biofilm activities of melittin and colistin, alone and in combination with antibiotics against Gram-negative bacteria. Journal of Chemotherapy. 2016;28(2):95–103.
7. Lo Sciuto A, Cervoni M, Stefanelli R, Mancone C, Imperi F. Effect of lipid A aminoarabinosylation on Pseudomonas aeruginosa colistin resistance and fitness. International Journal of Antimicrobial Agents. 2020;55(5).
8. Edrees NE, Galal AAA, Abdel Monaem AR, Beheiry RR, Metwally MMM. Curcumin alleviates colistin-induced nephrotoxicity and neurotoxicity in rats via attenuation of oxidative stress, inflammation and apoptosis. Chemico-Biological Interactions. 2018;294:56–64.
9. Goli HR, Nahaei MR, Rezaee MA, Hasani A, Kafil HS, Aghazadeh M. Emergence of colistin resistant Pseudomonas aeruginosa at Tabriz hospitals, Iran. Iranian Journal of Microbiology. 2016;8(1):62–9.
10. Kanamori H, Rutala WA, Weber DJ. The role of patient care items as a fomite in healthcare-associated outbreaks and infection prevention. Clinical Infectious Diseases. 2017;65(8):1412–9.
11. Levy SB. Antibiotic and antiseptic resistance: Impact on public health. Vol. 19, Pediatric Infectious Disease Journal. 2000.
12. Paul D, Chakraborty R, Mandal SM. Biocides and health-care agents are more than just antibiotics: Inducing cross to co-resistance in microbes. Ecotoxicology and environmental safety. 2019 Jun;174:601–10.
13. Cieplik F, Jakubovics NS, Buchalla W, Maisch T, Hellwig E, Al-Ahmad A. Resistance toward chlorhexidine in oral bacteria-is there cause for concern? Vol. 10, Frontiers in Microbiology. 2019.
14. Bauchner H, Fontanarosa PB, Livingston EH. Conserving supply of personal protective equipment - A call for ideas. JAMA - Journal of the American Medical Association. 2020;
15. Rutala WA, Weber DJ. Guideline for Disinfection and Sterilization of Prion-Contaminated Medical Instruments. Infection Control & Hospital

Epidemiology. 2010;31(2):107–17.

16. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Clinical and Laboratory Standards Institute. 2013;32(3):1–184.
17. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: An evaluation of three different screening methods. Indian Journal of Medical Microbiology. 2006;24(1):25–9.
18. GHELLAI L, HASSAINE H, KLOUCHE N, KHADIR A, AISSAOUI N, NAS F, et al. Detection of biofilm formation of a collection of fifty strains of Staphylococcus aureus isolated in Algeria at the University Hospital of Tlemcen. Journal of Bacteriology Research. 2014;6(1):1–6.
19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement Clinical and Laboratory Standards Institute. CLSI document M100-S16CLSI, Wayne, PA. 2015.
20. Szabó D, Bonomo RA, Silveira F, Pasculle AW, Baxter C, Linden PK, et al. SHV-type extended-spectrum beta-lactamase production is associated with reduced cefepime susceptibility in Enterobacter cloacae. Journal of Clinical Microbiology. 2005;43(10):5058–64.
21. Fazzeli H, Akbari R, Moghim S, Narimani T, Arabestani MR, Ghoddousi AR. Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens. Journal of Research in Medical Sciences. 2012;17(4):332–7.
22. S. FA, Buchanan RET, Gibbons NE, Bergey. Bergey's Manual of Determinative Bacteriology. Vol. 24, Taxon. 1975. 377 p.
23. Giannella M, Pascale R, Gutiérrez-Gutiérrez B, Cano A, Viale P. The use of predictive scores in the management of patients with carbapenem-resistant Klebsiella pneumoniae infection. Expert Review of Anti-Infective Therapy. 2019;17(4):265–73.
24. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen JA, Klugman K, et al. Access to effective antimicrobials: A worldwide challenge. The Lancet. 2016;387(10014):168–75.
25. Peterson E, Kaur P. Antibiotic resistance mechanisms in bacteria: Relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Frontiers in Microbiology. 2018;9(NOV).
26. Suleyman G, Alangaden G, Bardossy AC. The Role of Environmental Contamination in the Transmission of Nosocomial Pathogens and Healthcare-Associated Infections. Vol. 20, Current Infectious Disease Reports. 2018.
27. Rao SP, Rama PS, Gurushanthappa V, Manipura R, Srinivasan K. Extended-Spectrum Beta-Lactamases Producing Escherichia coli and Klebsiella pneumoniae: A Multi-Centric Study Across Karnataka. Journal of laboratory physicians. 2014 Jan;6(1):7–13.
28. Melake N, Mahmoud A, Elraghy N, Labib A, Hassan D, Elbrolosy A. Detection of *Klebsiella pneumoniae* carbapenemases and metallo-β-lactamases among *Klebsiella pneumoniae* isolates from hospitalized patients at Menoufia University Hospitals, Egypt. Menoufia Medical Journal [Internet]. 2016 Oct 1;29(4):801–11. Available from: http://www.mmj.eg.net/article.asp?issn=1110-2098
29. Nirwati H, Sinanjung K, Fahrunissa F, Wijaya F, Napitupulu S, Hati VP, et al. Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC Proceedings. 2019;13.
30. Diriba K, Kassa T, Alemu Y, Bekele S. *In Vitro* Biofilm Formation and Antibiotic Susceptibility Patterns of Bacteria from Suspected External Eye Infected Patients Attending Ophthalmology Clinic, Southwest Ethiopia. Iglewski BH, editor. International Journal of Microbiology [Internet]. 2020;2020:8472395. Available from: https://doi.org/10.1155/2020/8472395
31. Sanjee S Al, Hassan MM, Manchur M. In vitro biofilm formation by multidrug resistant clinical isolates of Pseudomonas aeruginosa. Asian Journal of Medical and Biological Research. 2018;4(1):105–16.
32. Seifi K, Kazemian H, Heidari H, Rezagholizadeh F, Saee Y, Shirvani F, et al. Evaluation of biofilm formation among klebsiella pneumoniae isolates and molecular characterization by ERIC-PCR. Jundishapur Journal of Microbiology. 2016;9(1).
33. Martin RM, Bachman MA. Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Frontiers in Cellular and Infection Microbiology. 2018;8(JAN):4.

34. Vandepitte J., Verhaegen J., Enbaek K, Rohna P, Piot P HC. Basic laboratory procedures in bacteriology. WHO. 2003.

35. Al-Ajilil EQ. Molecular Study of Extended Spectrum β-Lactamases and Metallo β-Lactamases Produced by Clinical Isolates of Acinetobacter baumannii and Pseudomonas aeruginosa. Al-Kufa University; 2013.

36. Bokaeian M, Zahedani SS, Bajgiran MS, Moghaddam AA. Frequency of PER, VEB, SHV, TEM and CTX-M genes in resistant strains of Pseudomonas aeruginosa Producing Extended Spectrum β-lactamases. Jundishapur Journal of Microbiology. 2015;8(1).

37. Hamdy Mohammed E sayed, Elsadek Fakhr A, Mohammed El sayed H, Al Johery S abd E, Abdel Ghani Hassanein W. Spread of TEM, VIM, SHV, and CTX-M *β*-Lactamases in Imipenem-Resistant Gram-Negative Bacilli Isolated from Egyptian Hospitals. Comi G, editor. International Journal of Microbiology [Internet]. 2016;2016:8382605. Available from: https://doi.org/10.1155/2016/8382605

38. Ahmed OI, El-Hady SA, Ahmed TM, Ahmed IZ. Detection of bla SHV and bla CTX-M genes in ESBL producing Klebsiella pneumoniae isolated from Egyptian patients with suspected nosocomial infections. Egyptian Journal of Medical Human Genetics. 2013;14(3):277–83.

39. Kiratisin P, Apisarnthanarak A, Laesripa C, Saifon P. Molecular characterization and epidemiology of extended-spectrum-beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrobial agents and chemotherapy. 2008 Aug;52(8):2818–24.

40. Stokes HW, Gillings MR. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. Vol. 35, FEMS Microbiology Reviews. 2011. p. 790–819.

41. Helal ZH, Khan MI. QacE and QacE?1 Genes and Their Correlation to Antibiotics and Biocides Resistance Pseudomonas aeruginosa. American Journal of Biomedical Sciences. 2015;52–62.

42. Shahat H, Mohamed H, Abd Al-Azeem M, Nasef S. Molecular Detection of Some Virulence Genes in Pseudomonas aeruginosa Isolated from Chicken Embryos and Broilers with regard to Disinfectant Resistance. SVU-International Journal of Veterinary Sciences. 2019;2(2):52–70.

43. Romão C, Miranda CA, Silva J, Mandetta Clementino M, De Filippis I, Asensi M. Presence of qacEΔ1 gene and susceptibility to a hospital biocide in clinical isolates of Pseudomonas aeruginosa resistant to antibiotics. Current Microbiology. 2011;63(1):16–21.

44. Abuzaid WZ, Sangid MD, Carroll JD, Sehitoglu H, Lambros J. Slip transfer and plastic strain accumulation across grain boundaries in Hastelloy X. Journal of the Mechanics and Physics of Solids. 2012;60(6):1201–20.