

Detection of Bacterial Resistance Genes from Neonatal's Incubators Environment at Selected Sites of Baghdad Hospitals

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ABSTRACT

Background: Nosocomial infections among neonates are responsible for 30–40% of death in resource constrained countries.

Objective: This study aimed to detect isolated bacterial resistance genes and to assess chlorine gas values, which were emitted during using Didecyl

dimethylammonium Chloride (DDAC) sterilizer in incubators of neonates at selected sites of Baghdad Hospitals.

Materials and Methods: Cl₂ was detected by a portable sensor device, antibiotic susceptibility test was determined using disc diffusion and detection of resistance genes by PCR technique.

Results: Presence of chlorine gas concentrations in the second and first sites during winter, which were higher than in summer and concentrations were highly significant ($P < 0.0001$) in second site and significant ($P < 0.05$) in first site. Third and fourth sites did not record any concentrations of chlorine. Pathogenic bacterial were isolated from these sites that included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aurogenosa* and *Enterobacter*. Antibiotic susceptibility test was done using disc diffusion method, which showed that all isolates were resistant to tested antibiotics specially to ampiclox and azithromycin ($P < 0.05$). Detection of resistance genes by PCR showed Gram negative isolate possess various percentages of genes including *TEM* (88.9%), *OXA-1* (77.8%), *CTX-M* (72.2%), *SHV* (66.7%) and Gram positive isolate possess *blaz* (100%). *aacA* and *mecA1* had same resistance genes prevalent (45.5%).

Conclusion: Using DDAC a sterilizer showed effectiveness in eliminating bacterial presence. Therefore, effective disinfectants must be used to eliminate microbes.

Keywords: Neonatal incubator, chlorine, Nosocomial infection.

INTRODUCTION

Early-onset infections (occurring in the first 72 hours of life), which are acquired at the moment of delivery, are separated from late-onset infections (occurring beyond 72 hours from birth), which are acquired at home or in a hospital. Cleaning is a crucial first step in any disinfection process since it helps to get rid of pathogens or greatly reduce their load on contaminated surfaces, however cleaning does not get rid of microorganisms⁽¹⁾.

Alkyl dimethyl benzyl ammonium chloride (ADBAC) and Didecyl dimethyl ammonium Chloride (DDAC) are registered active ingredients as antimicrobial products with the US Environmental Protection Agency (EPA) and other regulatory agencies around the world. Based on currently available data from EPA regulations (40 CFR 158.2230), the main hazard associated with DDAC and C12–C16 ADBAC is local effects through irritation⁽²⁾.

One of the most frequent occurrences in hospitalized neonates is bacterial bloodstream infection, which is primarily brought on by coagulase-negative *staphylococci*. The most frequent species that result in bacterial contamination of neonatal incubators are *S. aureus* and *S. saprophyticus*⁽³⁾. *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), *Klebsiella species*, *E. coli*,

Pseudomonas species, *Acinetobacter species*, and *Enterococcus species* are among the clinically significant possible pathogens⁽⁴⁾.

Resistance to antibiotics like penicillin, cephalosporin, carbapenem, and fluoroquinolones has also been significantly reported⁽⁵⁾. Based on comparisons of their amino acid sequences, the more than 350 different natural ESBL variants currently known have been divided into nine different structural and evolutionary families, including *TEM*, *SHV*, and *CTX-M*⁽⁶⁾.

The antibiotic resistance genes *mecA* and *aacA-D*, respectively, make the antibiotic groups methicillin, aminoglycosides, tetracyclines, macrolide-lincosamide-streptogramin B, macrolides, lincosamides, and streptogramin A all are susceptible to resistance⁽⁷⁾.

Therefore, this study aimed to detect of bacterial resistance genes isolated from incubators environment of neonates at selected sites of Baghdad hospitals.

METHODS

The Field of study

This study was performed at four pediatric hospitals in Baghdad two in AL-Karkh and two in AL-Rusafa. The samples were collected in tow season summer (June and July) and winter (December and January) for each pediatric neonatal hospital intensive care unit (NICU) the

Cl₂ were measured in these pediatric hospitals. In first site of study the incubators were sterilized in the sterilizing room but in the second pediatric hospital the incubators were sterilized at the same wards of NICU and opening the doors and windows of the room.

Both pediatric hospitals in AL Karkh using the Didecyldimethylammonium Chloride (DDAC) sterilizer one of the sterilization materials recommended by the Ministry of Health, which have proven their effectiveness in sterilization according to the Iraqi Central Organization for Standardization and Quality Control (Cosqc.IQ).

While, the other two pediatric hospitals in AL Rusafa were not using Didecyldimethylammonium Chloride sterilizer. They sterilized the incubators by ordinary sterilization materials such as alcohols. Swabs were taken from different parts of the incubators of all the pediatric hospitals that are in the study to investigate the microbial contamination in the incubator.

The total number of swabs reached 222 swabs for all study sites in summer and winter. The number of swabs for the first site was 25 swabs in summer and 25 swabs in winter, the number of swabs for the second site was 34 swabs in summer and 34 swabs in winter, the number of swabs for the third site was 32 swabs in summer and 32 swabs in winter and for the fourth site was 20 swabs in summer and 20 swabs in winter.

Detection of chlorine (Cl₂) concentration

Air samples in the (NICU) were measured to detect the concentration of Cl₂ by using a portable sensor devices for Cl₂ gas that has been calibrated by the manufacturer company (ALTAIR 2X, USA) to enable the sensor devices direct measuring the concentration of Cl₂ in part per million units (ppm).

Isolates of Bacteria

For bacterial isolation, 222 swabs were obtained from neonatal incubators that exist in neonatal intensive care unit at four hospital locations during the summer and winter. The isolated bacteria were cultivated and purified using specialized media provided by the company (Himedia/India), including nutrient, blood agar, MacConkey agar, brain heart infusion and Hi-chromogenic UTI agar. The cultures were incubated overnight at 37°C then kept under refrigerated condition.

Disc test for antimicrobial sensitivity

Phenotypic antibiotic resistance of bacterial isolates was made by disc diffusion method on Muller-Hinton agar using Amikacin (10µg/disc), Ampiclox (30µg/disc), Ampicillin (25µg/disc), Azithromycin (15 µg/disc), Ciprofloxacin (10 µg/disc), Gentamicin (10 µg/disc), Trimethoprim (10 µg/disc) and Vancomycin (30 µg/disc) (manufacturer Bioanalyse). The results were classified as susceptible, intermediate, or resistant in line with Clinical and Laboratory Standards Institute CLSI, (2020)

Molecular Techniques

DNA extraction

A commercial DNA extraction kit (gSYNCTM DNA extraction kit, Geneaid, Thailand) was used to extract DNA from bacterial isolates according to protocol of manufactures, the purity and concentration of DNA were determined by a nanodrop.

Primers

The primers used for amplification of resistance genes in gram-negative bacteria including *SHV*, *CTX-m*, *Amp-C*, *OXO1*, *OXO48*, *fim-H* and *markD* that were used for bacteria *Klebsiella*, which contain the genes that make up the biofilm. *aacA-D*, *mecA* and *blaz* were used for gram-positive bacteria that were listed in table (1).

Table (1): The primers used in the study

Type of bacteria	Genes	Primer	The sequence of Nucleotide (5' to 3')		Amplicon Size (bp)
G-ve	<i>blaSV</i>	<i>SHV</i>	F	AAGTCCACTATCGCCAGCAG	231 bp (8)
			R	ATTCAGTTCCGTTTCCAGCGG	
	<i>blaAmpC</i>	<i>AmpC</i>	F	ATTCGTATGCTGGATCTCGCCACC	395 bp (9)
			R	CATGACCCAGTTCGCCATATCCTG	
	<i>blaC-M</i>	<i>CTXm</i>	F	GACGATGTCACTGGCTGAGC	544 bp (10)
			R	AGCCGCCGACGCTAATACA	
	<i>blaoxA-1</i>	<i>OXA-1</i>	F	GATCGCATTATCACTTATGGC	454 bp (11)
			R	GGTTCTATTTGCTGTGAATCC	
	<i>blaoxA-48</i>	<i>OXA-48</i>	F	AATCATCAGGGGATTCTTCAG	481 bp (12)
			R	CCATAATCGAAAGCATGTAGC	
	<i>fimH</i>	<i>fimH</i>	F	ATGAACGCCTGGTCCTTTGC	688 bp (13)
			R	GCTGAACGCCTATCCCCTGC	
	<i>MrkD</i>	<i>mrkD</i>	F	CCACCAACTATTCCTCGAA	240 bp (14)
			R	ATGGAACCCACATCGACATT	
<i>blaTEM</i>	<i>TEM</i>	F	GAGTATCAACATTTCCGTGTC	848 bp (8)	
		R	TAATCAGTGAGGCACCTATCTC		
G+ve	<i>aacA-D</i>	<i>aacAD</i>	F	TAATCCAAGAGCAATAAGGGC	227 bp (14)
			R	GCCACACTATCATAACCACTA	
	<i>mecA</i>	<i>mecA</i>	F	GTGAAGATATACCAAGTGATT	147 bp (15)
			R	ATGCGCTATAGATTGAAAGGAT	
	<i>blaz</i>	<i>Blaz</i>	F	CAAAGATGATATAGTTGCTTATTCTCC	421 bp (16)
			R	TGCTTGACCACTTTTATCAGC	

Amplification of PCR

The amplification of DNA for the eleven genes had been done by the preparation of PCR reaction tube mixture, which contain primers (1 µl), extracted DNA template (5µl) and 5µl of Accu Power® PCR Premix (BioNeer, Korea) that were thawed at room temperature and nuclease free, which was added to complete the rest volume (25 µl) then vortexed to have homogenous contents.

The negative control tube contained all materials but did not contain the extracted DNA template where it was substituted by distilled water.

The thermal program was optimized and performed in master cycler (Eppendorf). Program for *SHV*, 3 min at 94 °C, then 28 cycles of 30 sec at 94 °C, 30 sec at 60 °C and 40 sec at 72 °C, then final elongation step at 72 °C for 5 min. For *TEM*, 3min at 95 °C, then 28 cycles of 30 sec at 95 °C, 30 sec at 57 °C and 40 sec at 72 °C, then final elongation step at 72 °C for 5 min. For *Amp*, 3 min at 94 °C, then 28 cycles of 30 sec at 94 °C, 30 sec at 55 °C and 40 sec at 72 °C, then final elongation step at 72 °C for 5 min. For *CTX-M*, 4 min at 94 °C, then 28 cycles of 40 sec at 94 °C, 30 sec at 55 °C and 30 sec at 72 °C then final elongation step at 72 °C for 5 min. For *OXA-1*, 4 min at 94 °C, then 35 cycles of 30 sec at 94 °C, 40 sec at 59 °C

and 25 sec at 72 °C then final elongation step at 72 °C for 4 min. For *OXA-48*, 5 min at 95 °C, then 35 cycles of 40 sec at 95 °C, 40 sec at 51.5 °C and 40 sec at 72 °C, then final elongation step at 72 °C for 5 min. For *Fim-H*, 5 min at 95 °C, then 35 cycles of 40 sec at 95 °C, 30 sec at 60 °C and 40 sec at 72 °C, then final elongation step at 72 °C for 5 min.

For *Mrk-D*, 3 min at 95 °C, then 35 cycles of 40 sec at 95 °C, 30 sec at 56.5 °C and 25 sec at 72 °C, then final elongation step at 72 °C for 4 min.

Multiplex PCR were done for *mecA* , *aacA-D* and *blaz* and their program as following: 5 min at 95 °C, then 35 cycles of 40 sec at 95 °C, 30 sec at 55.6 °C and 40 sec at 72 °C then final elongation step at 72 °C for 5min.

Ethical approval:

An approval of this study was obtained from the University of Baghdad Academic and Ethical Committee. Informed consent from all the patients was taken. This study was carried out in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for studies involving humans.

Statistical Analysis

IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) and GraphPad Prism version 8.0.0 (San Diego, California, USA) were used. The parameters were supplied as percentage, frequencies, and Pearson-Chi-square or two-tailed Fisher exact probability tests were used to determine whether there were significant differences between frequencies (p). To compare continuous variables (normally distributed) and to determine significant differences between means, the T-test and ANOVA probability were employed (asterisk denotes significant differences at p 0.05). Furthermore, the Person bivariate correlation was used to understand the relationship between various factors.

RESULTS

Chlorine concentration

The device that used to measure the concentration of polluting gases showed the presence of concentrations of chlorine gas, which is one of the components of DDAC used in sterilization. Figure (1) showed that there were significant differences in the measured chlorine (Cl_2) concentrations in the first and second sites, while the third and fourth sites did not record any chlorine concentrations. The highest concentration of chlorine was in the neonatal's incubators in the **second** site in winter, was 0.25 ppm, and the lowest concentration was in the first site in summer, was 0.12 ppm. Probability and the statistical analysis showed significant difference of Cl_2 (p -value < 0.0001) between the two sites and during two seasons.

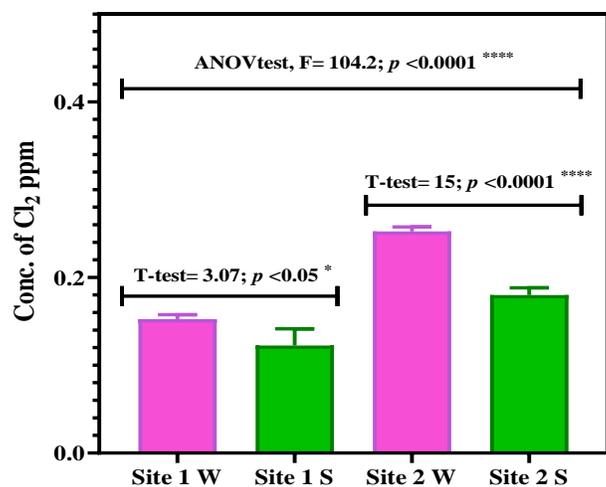


Figure (1): Mean of chlorine concentration among neonatals' incubators in site 1 and site 2 summer (S) and

during winter (W) seasons. P : T and one-way ANOVA test

Then, the comparison in chlorine concentrations in one site during the two seasons showed that the highest concentration of chlorine in the **first** site in winter was 0.15, while the lowest concentration in summer was 0.12, where the statistical analysis revealed significant differences between the two seasons in the **first** site at $T=3.07$ and $p < 0.05$. In the **second** site, in winter, the concentration of chlorine was higher than in summer as well, as is the case in the **first** site, but the concentrations of chlorine in summer and winter in the **second** site were higher than in the **first** site, where the highest in winter was 0.25 and the lowest in summer was 0.18 in the **second** site. This was what the results of the statistical analysis showed, the highest significance of the difference in chlorine concentrations between summer and winter in the **second** ($p < 0.0001$ and $T=15$).

Didcedimethylammonium Chloride (DDAC)

DDAC is a sterilizer used by 2 pediatric hospitals in AL Karkh for sterilization of the incubators and its equipments. The chemical composition is chloride (0.25 g), Benzalkonium chloride (0.5 g), auxiliary substances and Di water.

A quick ready-to-use, broad-spectrum sterilizer against germs (bacteria, viruses and mold) and is used for long-term sterilization. It is free of alcohol, phenols and formaldehydes. It is used for small surfaces, medical instruments, ICU, burn, emergency wards, dental clinics, medical equipment carts, tables, chairs, patient beds, robots, incubators, ambulances, laboratories, ultrasound equipment, and electronic washing machines. It is available in the form of a solution, soap and spray.

Types of bacteria isolated from the study sites

The third and fourth sites represent the two hospitals on Rusafa side that did not use the sterile material DDAC. Where the number of isolates in the first site was 25 in summer and 25 in winter swabs in which we did not find any microbial growth, and in the second site 34 swabs in summer and 34 swabs in winter also we did not find any microbial growth in it, while in the third and fourth site there were 29 isolates that were obtained from 104 swabs in the third and fourth sites during summer and winter. Figure (2) showed the types of bacteria isolated from newborn incubators in the two sites during the winter and summer seasons.

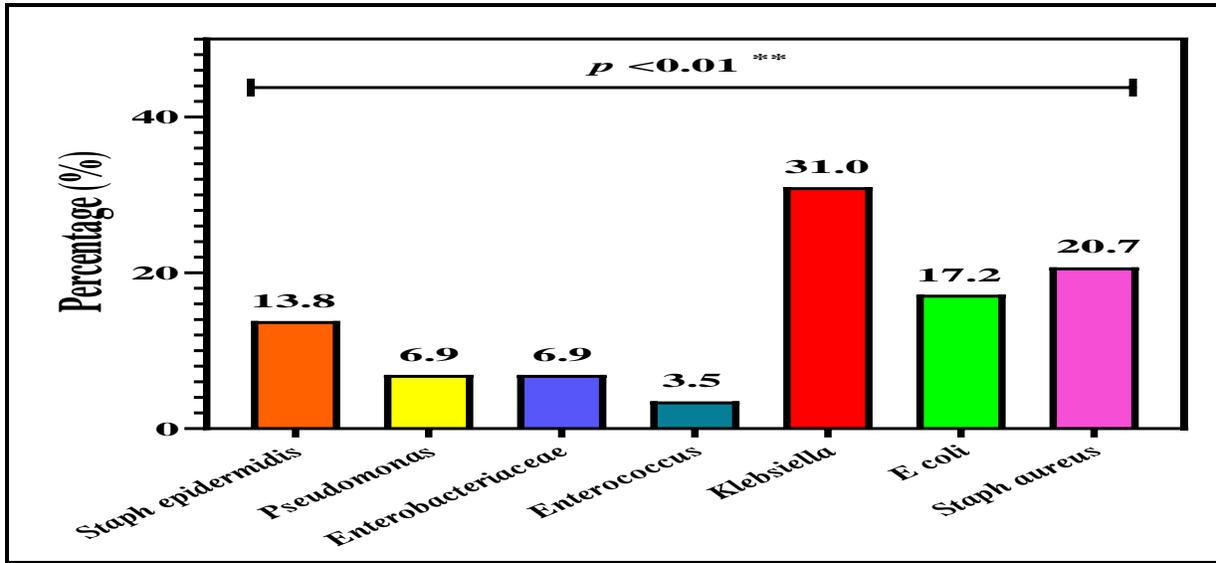


Figure (2): The percentages of Gram negative and positive bacteria present in neonatal incubators during Winter and Summer (p : Chi-Square test probability). The figure showed the percentages for the types of gram positive bacteria, which were *S. aureus*, *S. epidermidis*, *Enterococcus*, gram negative bacteria, *P. aeruginosa*, *klebsiella pneumonia*, *Enterobacter* and *E coli* where the highest percentage of gram negative bacteria was *K. pneumonia* (31%) and the lowest percentage for gram positive bacteria was *Enterococcus* (3.5%).

Test of antimicrobial susceptibility

The results of the antibiotic susceptibility test revealed that different isolates of bacteria had different resistance patterns to the tested antibiotics, as shown in figure (3). By using a disk diffusion test on Mueller-Hinton agar plates, the susceptibility test for 29 isolates (18 gram-negative isolates and 11 gram-positive isolates) was assessed against 8 antibiotics. The isolates showed resistance to ampiclox 44.7%, vancomycin 44.7%, azithromycin 39.5, ampicillin 36.8%, gentamicin 31.6%, amikacin 28.9%, trimethoprim 26.3% and ciprofloxacin 7.9%.

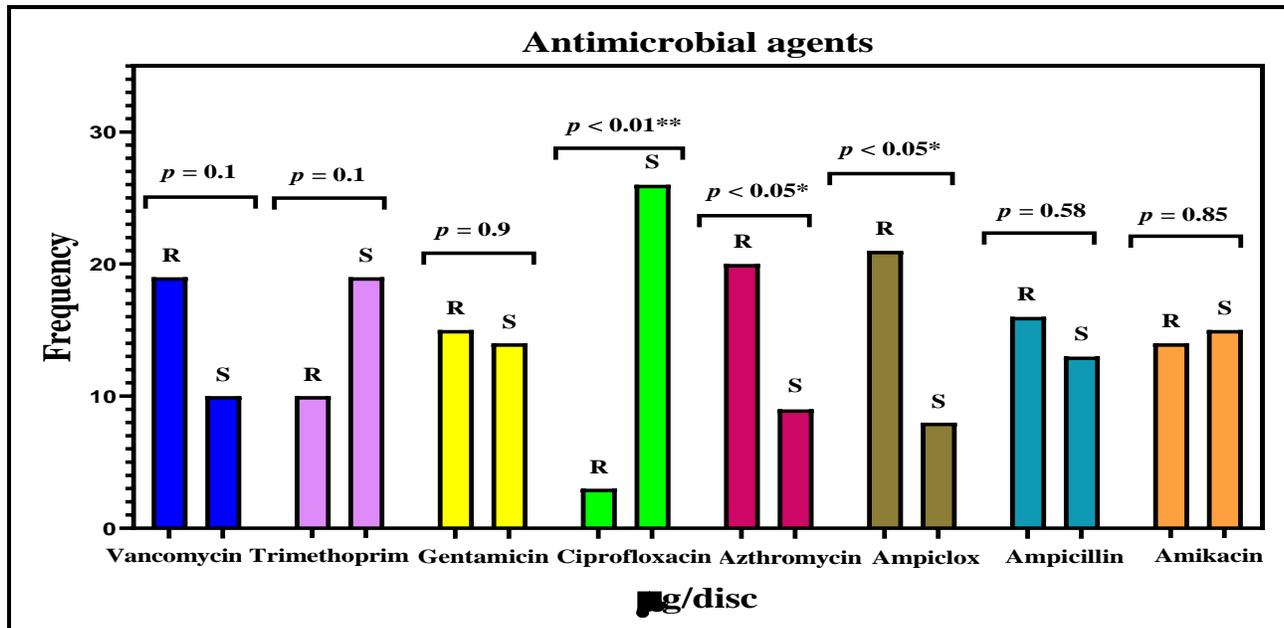


Figure (3): Frequencies of antimicrobial agents (10 – 30 μ g/disc) stratified to resistance (R) and sensitive (S) in Gram positive and negative bacteria present in neonatal incubators during Winter and Summer (p : Chi-Square test probability).

The statistical analysis showed that there were significant differences in the resistance of bacteria to some antibiotics such as ciprofloxacin ($p < 0.01$) as well as for ampiclox and azithromycin ($p < 0.05$). Table (3) showed the percentages of resistance for both positive and negative bacteria to the eight antibiotics, *E coli*, *P. aeruginosa* and *K. pneumoniae* had the highest range of resistance to studied antibiotics.

Table 3: Percentages of resistance ranges of studies pathogenic bacteria for antimicrobial agents

Gram negative isolates				Gram positive isolates		
<i>Klebsiella</i>	<i>E coli</i>	<i>P. aeruginosa</i>	<i>Enterobacter</i>	<i>Enterococcus</i>	<i>S. aureus</i>	<i>S. epidermidis</i>
37.5, 87.5%	62.5, 87 %	50 – 87, 5%	37.5%	37.5%	37.5%	37.5%

Molecular Analysis

Detection of Gram negative resistance Genes

Figure (4) showed the distribution percentages of studied genes in Gram negative bacteria where the results of the statistical analysis showed significant differences ($p < 0.01$). The prevalence of these genes were 88.9% for *TEM*, 77.8% for *OXA-1*, 72.2% for *CTX-M*, 66.7% for *SHV*, 38.9% for *Fim-H*, 27.8% for *mark-D* and 0% for *Amp*.

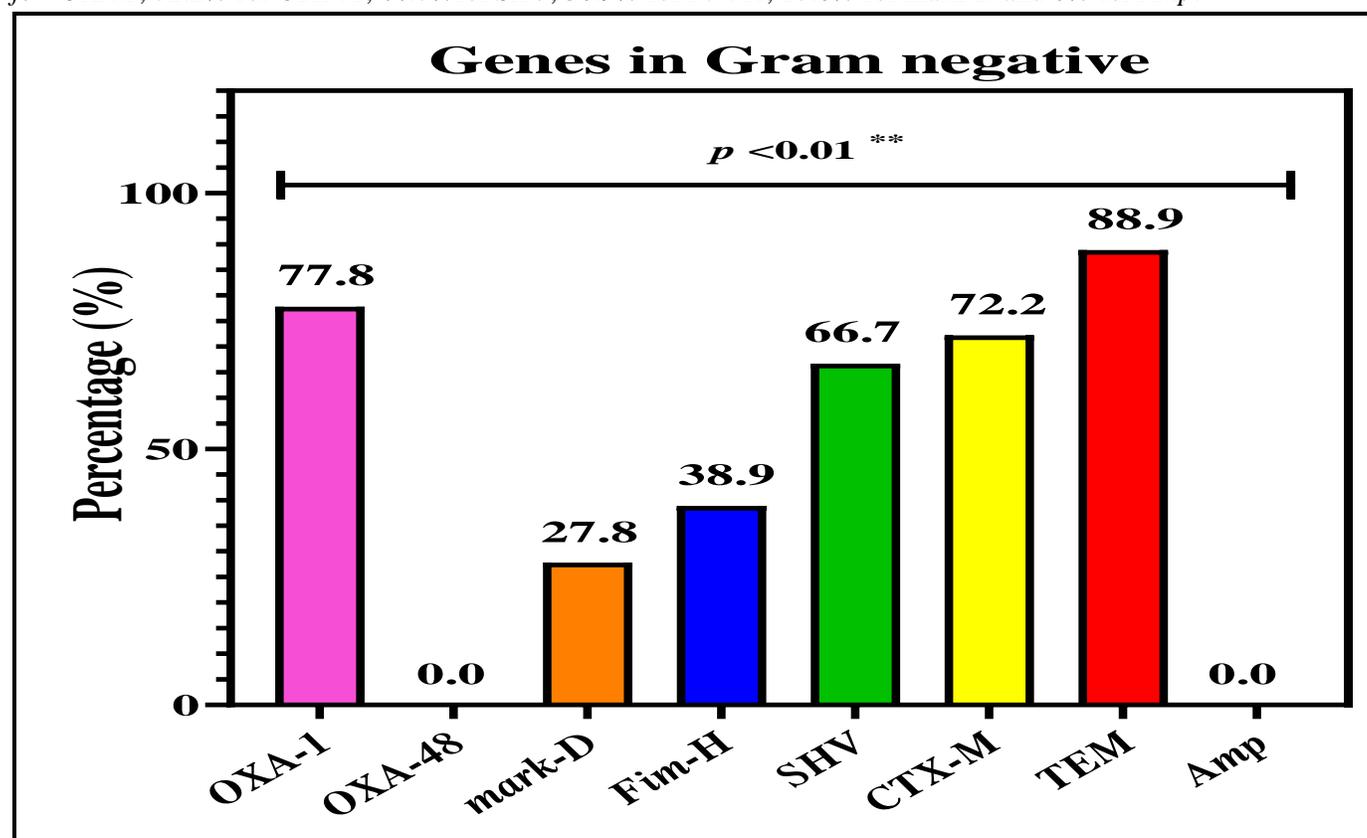


Figure (4): The percentages of antibiotics genes resistance in Gram negative bacteria present in neonatal incubators during Winter and Summer (p : Chi-Square test probability).

No spread of the *OXA-48* gene appeared in the types of negative bacterial isolates that were isolated from the incubators of newborns during the summer and winter seasons in the third and fourth sites. On the other hand, the results were unclear and inaccurate about the prevalence of the *Amp* gene in all isolates and the reason for the lack of clarity in the results of the *Amp*.

Detection of *blaOXA-1* gene

The current study showed that *blaOXA-1* gene (Figure 5) was found in 14 (77.8%) isolates included 6 (33.3%) of the *K. pneumonia* isolates, 5 (27.8%) of *E. coli*, 2 (11.1%) of *Enterobacter* and 1 (5.6%) of *Pseudomonas*. The total 14 isolates represented 77.8% from all types of isolates.

Detection of *Mark-D* and *Fim-H* genes

Health-care related infections are frequently caused by *K. pneumoniae*, which also has significant levels of drug resistance. The capacity of these bacteria to form biofilm is well recognized. This study found that *markD* was revealed in 5 (27.8%) isolates and *FimH* was revealed in 7 (38.9%) isolates of *K. pneumonia* as shown in figure (4).

Detection of *SHV*, *CTX-M* and *TEM* genes

Table (4) and figure (4) showed the prevalence rates of the three genes in various bacteria types isolated from the third and fourth sites during the summer and winter seasons. The results of PCR showed that the highest percentage of beta-lactamase genes were for the *TEM* gene (88.9%), *CTX-M* gene 72.7%, and *SHV* gene 66.7%. The most prevalence of these genes were found in *K. pneumonia* 44.45 %, 33.55 % and 33.35 % respectively.

Table 4: Frequency of *SHV*, *CTX-M* and *TEM* genes among bacterial isolates according to PCR analysis

Bacteria	Type of Resistant genes No. (Percentage)		
	<i>SHV</i>	<i>CTX-M</i>	<i>TEM</i>
<i>K. pneumonia</i>	6 (33.35 %)	5 (33.55 %)	8 (44.45 %)
<i>E coli</i>	4 (22.30%)	5 (27.96%)	4 (22.23%)
<i>Pseudomonas</i>	2 (11.12%)	2 (11.18%)	2 (11.11%)
<i>Enterobacter</i>	-	-	2 (11.11%)
The total Percentage (%)	12 (66.77%)	3 (72.69%)	16 (88.90%)

Detection of Gram positive resistance Genes

The current study demonstrated the prevalence of the three gram positive resistance genes in *S. aureus*, *S. epidermidis* and *Enterococcus* isolates as shown in figure 5.

The results in figure 10 illustrated the distribution percentages of gram positive resistance genes from the third and fourth sites and during the summer and winter seasons. The *blaz* was the highest gene prevalence rate which was 100% in all isolates. While, *mecA* and *aacA* were lowest and they had the same prevalence rate which was 45.5%. Statistically, there were no significant differences in the percentages of the prevalence of resistance genes among Gram positive isolates but there were significant differences in prevalence percentages of resistance genes for Gram positive isolates with prevalence percentages of resistance genes for Gram negative isolates at $p < 0.01$ as shown in figure (4).

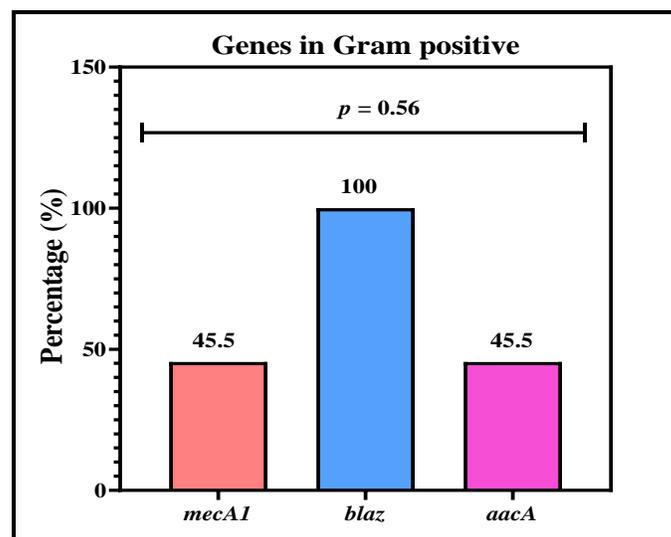


Figure 5: The percentages of antibiotics genes resistance in Gram positive bacteria present in neonatal incubators during Winter and Summer (p : Chi-Square test probability).

Table5: Frequency of *mecA*, *blaz* and *aacA* genes among bacterial isolates according to PCR analysis

Bacteria	Type of Resistant genes No. (Percentage)		
	<i>mecA</i>	<i>blaz</i>	<i>aacA</i>
<i>S. aureus</i>	3 (27.3%)	6 (54.54%)	3 (27.3%)
<i>S. epidermidis</i>	2 (18.2%)	4 (36.36%)	1 (9.1%)
<i>Enterococcus</i>	0	1 (9.09%)	1 (9.1%)
The total Percentage (%)	5 (45.5%)	11 (100%)	5 (45.5%)

DISCUSSION

The current study results showed that the concentrations of chlorine which is one of DDAC component, a substance recommended by the Ministry of Health, may be the cause of the high chlorine concentrations found in the first and second sites of the two hospitals situated in Al-Karkh side. DDAC is a chemical anti-septic that is used in many biocidal applications for its rapid killing ability⁽¹⁷⁾. When used in aerosol form, DDAC can potentially be exposed through the respiratory system, especially for those utilizing the product at work. During the dry winter months in South Korea, DDAC-containing humidifiers are heavily utilized to provide humidity⁽¹⁸⁾.

Pregnant women, their infants, and postpartum women who were receiving care in hospitals or indoor facilities became the principal victims of exposure to biocides as a result of this extended exposure to DDAC. Unfortunately, this exposure led to an unidentified interstitial lung disease in some individuals, which proved to be deadly⁽¹⁹⁾. Regarding the results of the percentages of resistance ranges for both Gram- positive and -negative bacteria to the eight antibiotics, *E. coli*, *P. aeruginosa* and *K. pneumoniae* had the highest range of resistance while the range of resistance obtained from study carried by **Sabir et al.**⁽²⁰⁾ showed that the resistance rate of *E. coli* toward gentamycin was 59.8%, amikacin was 12.7% and ciprofloxacin was 54.2%, which was lowest than the result in current study.

The percentages of resistance ranges in a study by **Alzaidi**⁽²¹⁾ reported that *P. aeruginosa* showed antibiotic resistance rate to gentamycin of 61.1%, ciprofloxacin was 44.4% and amikacin was 33.3%. The stronger resistance to beta-lactam antibiotics because of the overproduction of beta lactamase caused by resistance genes and mutational mechanisms, *P. aeruginosa* exhibited stronger resistance to beta-lactam antibiotics compared to non-beta-lactam antibiotics⁽²²⁾. For *K. pneumoniae* isolates there are many reports that were compatible or close to the results obtained from our study.

Hammoudi⁽²³⁾ showed that the resistance rate of *K. pneumoniae* isolates toward gentamicin was 78% and amikacin was 87 %⁽²³⁾. Another study by **Ahmed et al.**⁽²⁴⁾ found the resistance rate of *K. pneumoniae* for ciprofloxacin was 85%. *S. epidermidis* in this study showed 37.5% resistance rate, which disagrees with **Aku1 et al.**⁽²⁵⁾ results where they showed that the resistance rate of *S. epidermidis* were 100% to ampicillin and 57% to gentamicin as well as *S. aureus* was resistant 100% to ampicillin and 50% to gentamicin. Methicillin-resistant *S. aureus* (MRSA) strains evaluated in the study by **Khosravi et al.**⁽²⁶⁾ showed a high resistance rate to aminoglycoside

antibiotics, ranging from 77.6% in amikacin to 96.3% in neomycin. The distribution for percentages of antibiotics genes resistance in Gram negative bacteria present in neonatal incubators during winter and summer were unclear and inaccurate about the prevalence of the *AmpC* gene in all isolates. The reason for the lack of clarity in the results of the *AmpC* for this study may be due to prevalence of ESBL production varies according to species, geographical areas, variations in infection control programs, different patterns of empiric antibiotic regimens and even over time. Moreover, selective pressure caused by the overuse of cephalosporins in some countries leads to the emergence of increasing rates of ESBLs production⁽²⁷⁾.

In the detection of *blaOXA-1* gene the results showed that the prevalence of the resistance gene *OXA-1* for this study was 77.8%, which is close to the results of **Shahi**⁽²⁸⁾ in the spread of the same gene in isolates of *K. pneumoniae*, while spread of *OXA-48* was 66.67%⁽²⁸⁾. Moreover, the distribution of percentages for *Mark-D* and *Fim-H* antibiotics genes resistance in this study found that *markD* was revealed in 27.8% isolates and *FimH* was revealed in 38.9% isolates of *K. pneumoniae*. The results of this study do not agree with the study of **Kimia et al.**⁽²⁹⁾, while agree with **Duran et al.**⁽³⁰⁾ where they found the percentage of the two genes *Mark-D* and *Fim-H* was 100%⁽³⁰⁾. The difference in the percentages of *Mark-D* and *Fim-H* genes for this study, with previous studies, may be due to the presence of a high level of genetic diversity among strains of *K. pneumoniae*⁽³¹⁾.

As *K. pneumoniae* isolate may lose or form these genes depending on their need and the nature of the environment in which they live. the frequency of *SHV*, *CTX-M* and *TEM* genes among bacterial isolates according to PCR analysis the results observed the highest percentage of beta-lactamase genes were for the *TEM* gene (88.9%), *CTX-M* gene (72.7%), and *SHV* gene (66.7%). The most prevalence of these genes were found in *K. pneumoniae* (44.45 %, **33.55 %** and **33.35 %**) respectively. *Klebsiella* is one of the types of bacteria responsible for infection in hospitals because it possesses all resistance genes in almost all its species, and therefore it was the most prevalent in the sites of this study. More critically, the existence of β -lactamases genes as demonstrated by PCR does not necessarily indicate their production because it depends on environmental factors such as the presence of antibiotics⁽³²⁾.

Meanwhile, in a recent investigation in India utilizing specific primers for *blaCTX-M*, only 75.2% of ESBL isolates for one or more genes were found. The distribution of percentages for antibiotics genes resistance

in Gram positive bacteria present in neonatal incubators during winter and summer which *blaZ* was the highest gene prevalence rate which was 100% in all isolates. While *mecA* and *aacA* were lowest and they had the same prevalence rate, which was 45.5%. While, the distribution rate of *aacA* and *blaZ* in the study of **Duran et al.** ⁽³³⁾ showed that the distribution of antibiotic resistance genes *mecA* and *blaZ* among coagulase negative staphylococci strains isolated from human clinical illnesses was 29.60% and 9.40%. Also, **Pourmand et al.** ⁽³⁴⁾ reported that 95.8% of *S. epidermidis* isolates harbored *mecA*. This study data was significantly higher than **Ghazvini et al.** ⁽³⁵⁾ results, which reported only 10% *mecA* distribution. The difference in the prevalence of positive resistance genes may be due to the ability of positive isolates to lose and gain them in order to provide them with appropriate environmental condition.

CONCLUSION

Use of a sterilizer DDAC showed its effectiveness in eliminating the bacterial presence, and it is a double-edged sword, as it must be used within the recommended concentrations because high concentrations cause irritation. The third and fourth sites of incubators that did not use effective disinfectants, such as DDAC used in the first and second sites of the study, recorded the presence of bacteria responsible for nosocomial infection in hospitals, and therefore the newborn is susceptible to infection in the hospital and injury occurs inside the incubator. Therefore, effective disinfectants must be used to eliminate microbes, and continuous monitoring of the incubator's cleanliness to try to provide a safe environment for the newborn.

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