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Detection study of Some Virulence factors of Respiratory pathogens isolated from Electrical Generators Workers

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ABSTRACT

The respiratory tract has sites which are potentially inaccessible to microbes. But, microbes when enter, they caused Respiratory Tract Infections, which in turn become more resistant for treatment due to improper use of antibiotics, in addition they prescribed by unspecialized persons. This led to create microbes which are different from the original microbes by gaining new strategies for infection and persistence, antibiotic resistance, different virulence factors, various phenotypes and adaptive changes to cause host damage and disease. The generators produce harmful exhaust emissions such as; carbon monoxide, nitrogen oxides, hydrocarbons, particulate matter and sulfur dioxide. These exhaust emissions are harmful and can not be ignored especially for all the living creatures and surrounding environment. 184 Sputum samples from both workers and non-workers in electric generators who suspected had lower respiratory tract infection were collected. All samples were cultured and identification of bacteria and antimicrobial sensitivity tests were done by using a Vitek 2 system. Detection of some virulence factors was done. Out of 184 samples, 27 samples (14.67 %) produced a significant growth, The predominant bacteria was *Klebsiella* spp. The highest No. of Resistant bacterial isolates of Workers in electrical generators were for: Ampicillin, Cefazolin and Nitrofurantion. There were different levels of antibiotic resistance in both groups, in addition to different virulence factors presence (e.g. capsule, Lipase, protease and ESBLs, Efflux pump and string test). Statically, there were significant difference between both groups at ($p<0.05$) for biofilm formation.

1. Introduction

The Respiratory Tract is free from microorganisms, but when Immune System of host give up of fighting them, these microorganisms win the battle and colonize it. In addition to these infectious microorganisms, there are Opportunistic microorganisms which change their mechanisms from normal flora of adjacent areas into disease-causing microorganisms and colonize it also [1]. There are many factors that predispose for Respiratory Tract Infections (RTIs) e.g. immunodeficiency diseases, cardiovascular disease, liver disease, cancer, inhalation therapy, asthma, diabetes, overweight, dementia, smoking, alcoholism [2]. Antibiotic

resistance of pathogenic bacteria has become a common scenario of RTIs causes, which could be resulted from forming biofilm, Efflux pump formation, selective pressure, production of degrading enzymes, gaining resistance genes by plasmids, Transposons, etc… [3], [4].

Although the benefits of generating electricity but generators had its own harmful effects due to emissions production through the exhaust pipes or other system into the surrounding, which produced as a result of combustion of fuels, natural gas, gasoline and petrol [5]. Carbon dioxide, water and nitrogen from air are the major products of a complete combustion of petroleum-based fuels in generators. A very small percentage of the nitrogen is changed to nitrogen oxides and some other nitrated hydrocarbons. In addition to: Carbon monoxide (CO), hydrocarbons (HC), and oxides of nitrogen (NOx), particulate matter and sulfur dioxide. These emissions are harmful to humans and surrounding environment [6]. A researcher found that generators produce emissions that spread surrounding environment, here were many harmful exhaust emissions cause health problems (e.g fatigue, headache, respiratory tract infection, poisoning by carbon monoxide, bleeding, depression, lowered immune system and cancer among many surrounding humans [6]. The study aimed to detect and evaluate antimicrobial susceptibility profiles of bacteria from sputum of workers and non-workers in electrical generators suspected with LRTIs. Also distribution of some virulence factors (including Capsule, Protease, Lipase, ESBLs, Efflux pump, String test and serum resistance) and biofilm formation of bacterial isolates.

Samples Collection

Sputum Samples were collected from persons who were infected by respiratory tract infections who were working at electrical generators in Baghdad- Iraq and compared with persons who had also respiratory tract infection but not working at electrical generators, more details were found in a previous study.

Twenty seven Bacterial isolates from workers and non-workers in Electrical generators who had Respiratory trat infections were identified and detected their Sensitivity to antibiotics utilizing a Vitek 2 system according to the manufacturer's instructions (BioMerieux, France).The antibiotics included in the present study for Gram Negative bacteria were: Ampicillin (AMP), Amoxicillin/Clavulanic acid (AMC), Piperacillin/Tazobactam (TZP), Cefotaxime (CTX), Ceftazidime (CAZ),Cefepime (CPM), Ertapenem (ETP), Imipenem (IPM), Mero penem (MEM), Amik acin (AMK), Gentamicin (GEN), Ciprofloxacin (CIP),Norfloxacin (NOR), Fosfomycin (FOS), Nitrofurantion (F), Trimethoprim/Sulfamethoxazole (SXT), Cefazolin (CZ), Cefoxitin (FOX), Ceftriaxone (CRO), Levofloxacin (LVX) and Tigecycline (TCG). Antibiotics used for Gram Positive bacteria were: Benzyl Penicillin (P), Oxacillin (OXA), Gentamicin (GEN), Ciprofloxacin (CIP), Moxifloxacin (MXF), Clindamycin (CM), Linezolid (LZD), Teicoplanin (TEC), Vancomycin (VA), Tetracyclin (TE), Fosfomycin (FOS), Fusidic acid (FD), Rifampicin (RA), Trimethoprim/ Sulfamethoxazole (SXT), Ampicillin (AMP), Gentamicin (GEN), Streptomycin (S), Erythromycin (E) and Doxycyclin (DOX).

Oxidase and Catalase tests

Catalase test and Oxidase test were done as mentioned by [7], [8] respectively.

Detection of Some Virulence factors and Bacterial products

1-Capsule

Capsule was detected by using India ink [9].

2- Protease enzyme

Bacterial isolates were cultured on the Skim milk agar medium. After incubation at 37ºC for 2-3 days, Clear

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zones of casein hydrolysis around the bacterial growth was a positive result [10].

3- Lipase enzyme

Bacterial isolates were cultured on Tributyrin agar, then incubated at 37o C for (24-48) hours. A positive result were recorded by clear zone of lipolysis around the single line of culture [11].

4- Extended Spectrum Beta-Lactamase (ESBLs) enzymes production test

Double-Disk Synergy Test (DDST) DDST was used to detect ESBL production [12], This was done by using a sterile cotton swab to inoculate Mueller-Hinton agar plates by the isolates (equal to 0.5 of McFarland). After that, an Augmentin disk (AMC) (composed of 20 µg amoxicillin and 10 µg clavulanic acid) on the center of the cultured plate, then was put together in addition to two disks of 3rd generation cephalosporin e.g. Cefotaxime (CTX) (30 µg) and Ceftazidime (30 µg). These disks were away 2cm away from the disk of AMC (start from center to center), then incubated at 37°C for 24 hr. The production of ESBLs from the bacterial isolates was noticed by an increasement in the zone of inhibition of any of the two antibiotic disks towards AMC

5-Detection of Efflux Pump activity

The Ethidium Bromide (Et Br)-agar cart wheel method mentioned by [13] was done to detect the activity of efflux pump of both isolates of workers and non-workers in Electric generators. Bacterial cell suspension (106) cells /ml was cultured on plates of Mueller–Hinton agar including (0, 0.5, 1, 1.5, and 2) mg/l of EtBr , incubate at 37 °C for 24 hr. After incubation, UV transilluminator was used to examine the plates for fluorescence, fluoresced isolates at the minimal concentration of EtBr were read as not containing active of efflux pumps, whereas those not fluorescent isolates had an active efflux pumps.

6-Determination of the hyper virulent K. pneumoniae phenotype (String test)

K. pneumoniae isolates which were isolated from sputum samples of both workers and non-workers in Electric generators were cultured on blood agar medium plates, incubated at 37°C for 24 hr. The positive result were recorded when a viscous string more than 5 mm by using a conventional bacteriology loops in order to identify the hyper virulent Klebsiella pneumoniae phenotype [14].

7- Serum Resistance test

The serum bactericidal test was done as mentioned by [15]. The bacterial isolates were cultured on plates of Blood agar, incubate at 37°C for 24 hr to collect a single colony for culturing in 5 ml of Brain Heart Infusion Broth for (4–6) hr at 37°C. After that, culture broth were centrifuged at 200 rpm until the optical density (OD at 630) reach 0.6, Then bacteria was diluted to (106) cells/ml in Normal saline. Then, 50 µl of bacterial suspensions were mixed with 150 µl of serum (taken from healthy human) in a centrifuge tube (2 ml), mixed, then incubated at 37°C for (0, 1, 2, and 3) hr, respectively. Tubes were centrifuged at 200 rpm, then 5 µl of bacterial solution was drawn at each recorded time and 100 times were diluted with normal saline. Finally, 50 µl drawn and inoculate on Muller Hinton agar plates for count the bacterial colonies. Results were interrupted by the inoculum percentage of viable counts and were graded as highly susceptible (grade 1 or 2), intermediately susceptible (grade 3 or 4), or resistance (grade 5 or 6).

8-Biofilm formation assay

Microliter Plate Assay was performed for biofilm formation according to [16].

2. Findings and Discussion

In a previous study ,184 sputum samples from men who were working in electrical generators (mean

duration of work (9.2±1.1 years) and age (39.7±1.86 years) in Baghdad-Iraq were dealt as mentioned by the standard microbiological methods, in microscopical examination of the smear of sputum had <10 squamous epithelial cells and 25> pus cells(or leucocytes) for each low power field recorded the rightness of the specimen, confirmed it was not saliva contaminated [17], leading to identify 27 samples (14.67 %) which recorded a significant growth of microbes on culture media, distributed to 25 isolate (92.59%) of Gram negative bacteria and 2 isolate (7.41%) of Gram positive bacteria. *Klebsiella* spp. were the predominant bacteria *Klebsiella* spp. followed by other genus as shown at Table (1) and Figure (1).

Table (1) Distribution of Bacteria isolated from workers in Generators

Bacterial isolate	No. (%)
Klebsiella pneumoniae	11 (40.74)
Klebsiella oxytoca	2 (7.40)
Burkholderia cepacia	3 (11.11)
Enterobacter cloacae	2 (7.40)
Enterobacter aerogenes	1 (3.7)
Serratia marcescens	1 (3.7)
Serratia ficaria	1 (3.7)
Stenotrophomonas malltophilia	1 (3.7)
Proteus mirabilis	1 (3.7)
Escherichia coli	1 (3.7)
Enterococcus faecalis	1 (3.7)
Pseudomonas aeruginosa	1 (3.7)
Staphylococcus aureus	1 (3.7)

Table (2) Distribution of Bacteria isolated from non-workers in Generators

Bacterial isolate	No. (%)
Klebsiella pneumoniae	8 (29.63)
Klebsiella oxytoca	1 (3.7)
Acinetobacter baumannii	8 (29.63)
Pseudomonas aeruginosa	6 (22.22)
Enterobacter cloacae	1 (3.7)
Serratia marcescens	1 (3.7)
Burkholderia cepacia	1 (3.7)
Proteus mirabilis	1 (3.7)

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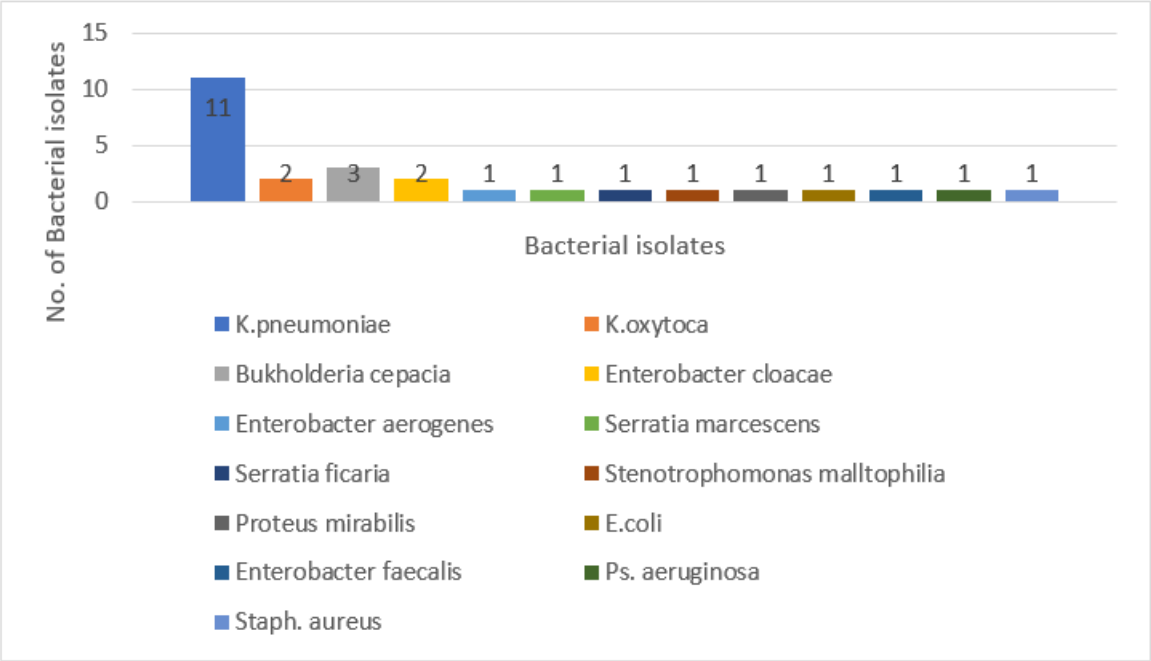

Figure (1) Distribution of Bacteria isolated from workers in Generators

The Sputum samples of non-workers in electrical generators was also confirmed diagnosis in order to get 27 bacterial isolate to use them for comparison as control. The predominant bacteria was also *Klebsiella* spp. followed by other genus as mentioned at Table (2) and Figure (2).

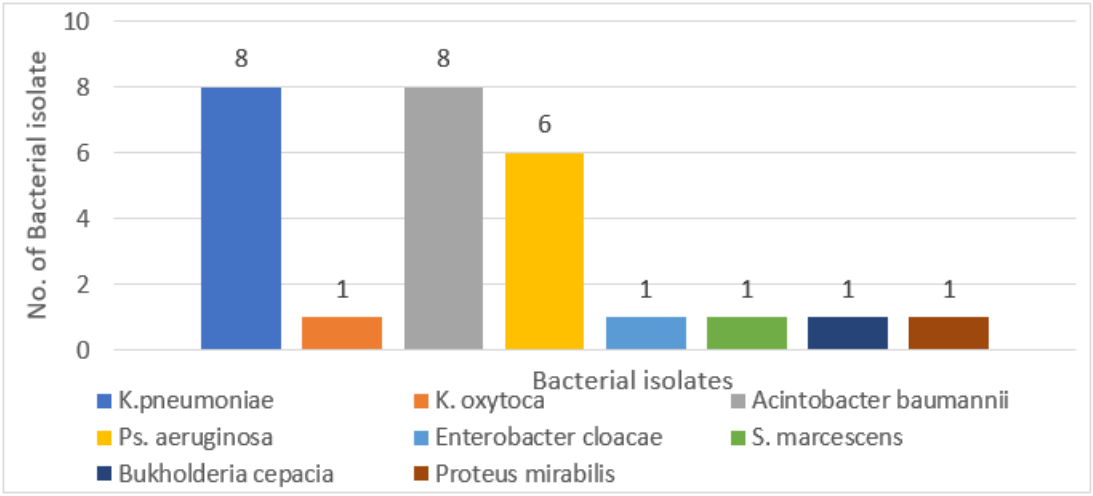

Figure (2) Distribution of Bacteria isolated from non-workers in generators

Smoking, alcohol consumption and aging are some of factors that predisposing for occurrence of LRTI in men [18].

The study showed compatibility with another study [19], when his results on LRTIs showed a high percentages of incidence for Gram negative bacteria compared with Gram positive bacteria. Our findings were parallel with [20], when found in his study that highest percentage of isolation were for Gram-negative bacteria (72.6%), followed by (23.1%) for Gram-positive bacteria, and (4.3%) for fungi, he stated that the main causing pathogens of LRTIs were (27.4%) for *K. pneumoniae*, (17.9%) *E. coli*, (10.3%) *P. aeruginosa*, (12.0%) *Staphylococcus aureus*, and (9.4%) *Streptococcus pneumoniae* respectively.

The study showed high incidence of infections by Gram negative bacteria compared with Gram positive bacteria, this could be explained the antibiotics used for treatment of Gram positive bacteria were more available and prescribed by unspecialized persons, even for some simple cases of infections, in addition, to absence of choosing the right antibiotic by many doctors leading into antibiotics overuse especially penicillins and cephalosporins [21].

Between the major common bacteria causing dangerous infections in the respiratory tract in human and animal is *Klebsiella* spp., This a result for its virulence factors (e.g. O-LPS, factors of adherence, antigens of capsule, and siderophores, which play a major role in its survival in harsh niches) [22].

The genome of *K.pneumoniae* had many virulence genes, e.g. muco viscous -associated gene (*magA*), uridine di phosphate galact uronate 4-epimerase gene (*uge*), and uptake system gene of iron (*kfu*), which are encoding for invasion ,colonization and pathogenic [23]. In addition, *Klebsiella* is resistant for phagocytosis and the serum bactericidal activity by its mucous capsule [24].

The highest No. of Resistant bacterial isolates of Workers in electrical generators were for: Ampicillin, Cefazolin and Nitrofurantion.

From the other side, the most powerful antibiotics were: Cefotaxime, Fosfomycin, Ertapenem, Tigecyclin in the first degree because only two isolates showed resistance for each one of them. In the second degree, the Antibiotics Meropenem, Amikacin and Levofloxacin were resisted by four isolates only as shown at Table (3).

Table (3) Antibiotic Resistance pattern of bacterial isolates isolated from workers in electrical generators

Antibiotics	Resistant Bacterial isolates No. (%)
Ampicillin	16 (59.25)
Cefazolin	12 (44.44)
Nitrofurantion	11 (40.74)
Cefatazidime, Imipenem, Gentamicin, Ceftriaxone, Trimethoprim/Sulphamethoxazole	8 (29.62)
Norfloxacin	7 (25.92)
Cefeipime,Ciprofloxacin, Cefoxitin	6 (22.22)
Amoxicillin ,Piperacillin/Tazobactam	5 (18.51)
Meropenem , Amikacin, Levolfloxacin	4 (14.81)
Cefotaxime, Fosfomycin, Ertapenem, Tigecyclin	2 (7.4)

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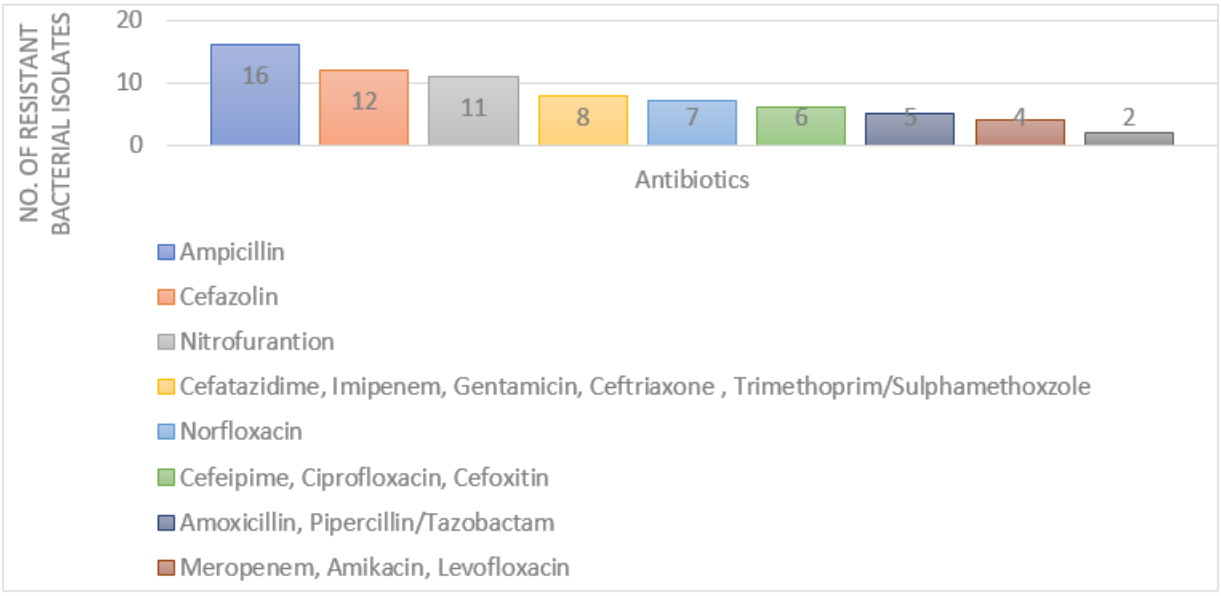

Figure (3) Antibiotic Resistance pattern of bacterial isolates isolated from workers in electrical generators

The bacterial isolates isolated of non-workers in generators showed high resistance to Cefotaxime in the first degree, to Amikacin, Meropenem and Trimethoprim/Sulphamethoxazole in second degree, to ciprofloxacin in third degree. In addition to that the highest effective antibiotics were Fosfomycin, Tigecyclin, Cefazolin, Cefoxitin and Ceftriaxone as shown at Table (4) and Figure (4).

Table (4) Antibiotic Resistance pattern of bacterial isolates isolated from non-workers in electrical generators

Antibiotics	Resistant Bacterial isolates No. (%)
Cefotaxime	16 (59.25)
Amikacin, Meropenem , Trimethoprim/Sulphamethoxazole	13 (48.14)
Ciprofloxacin	12 (44.44)
Imipenem,	10 (37.03)
Piperacillin/Tazobactam, Cefotazidme	9 (33.33)
Cefeipime, Gentamicin	7 (25.92)
Ampicillin, Amoxicillin/Clavulanic acid, Levofloxacin	6 (22.22)
Norfloxacin	5 (18.51)
Ertapenem, Nitrofurantion	3 (11.11)
Cefazolin, Cefoxitin, Ceftriaxone	2 (7.4)
Tigecyclin	1 (3.7)
Fosfomycin	0 (0)

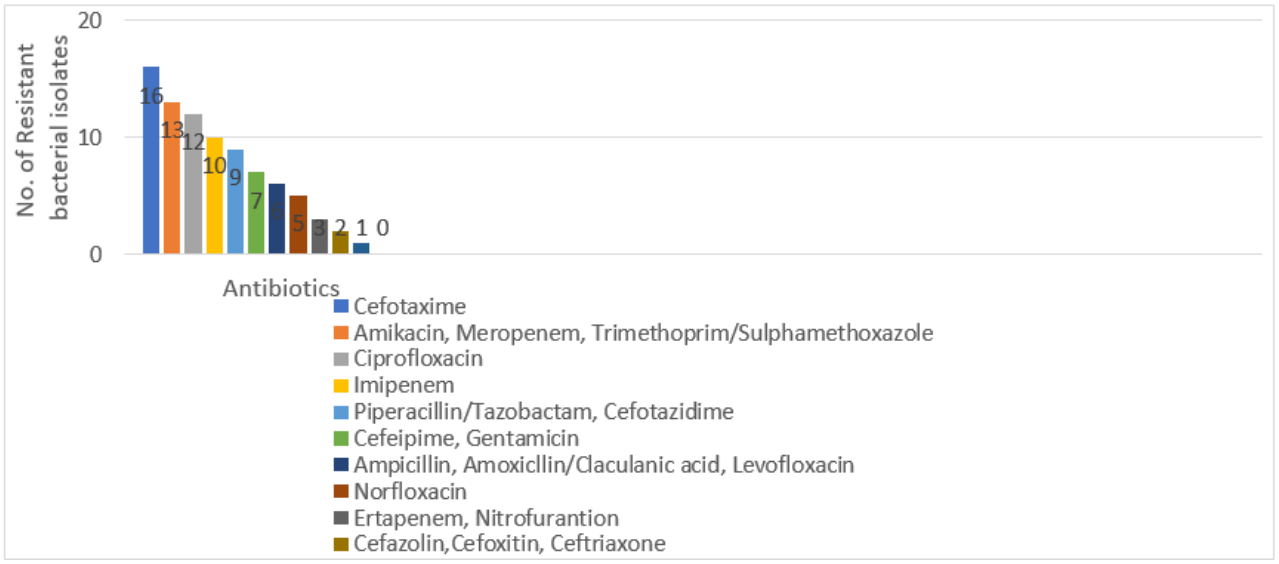

Figure (4) Antibiotic Resistance pattern of bacterial isolates isolated from non-workers in electrical generators

In the present study, resistance of the Gram negative bacteria isolated from non-workers in electrical generators to cephalosporins was high, which may be explained of widespread use of them also (26-a). Amikacin was so active antibiotic against the major Gram-negative bacteria isolated from workers in electrical generators of this study, but it had higher toxicity to the kidney and poor action against some antibiotic resistant strains [25].

Oxidase test for bacterial isolates of workers in electrical generators was done, only 4 out of 27 isolate showed positive result, in comparsion with 7 only out of 27 isolate of non-workers in electrical generators. Catalase test showed positive results for all isolates for both workers and non-workers in electrical generators. Catalase-positive pathogens use catalase to deal with the peroxide radicals, so they could survive unharmed inside the host [26].

Capsule production test showed positive results for 20 isolates of workers in electrical generators, in compare with 18 isolates of non-workers in electrical generators as shown at Table (5). Klebsiella pneumoniae had a capsule composed of acidic complex polysaccharides and responsible of their pathogenicity, it defends bacteria from action of phagocytosis and bacteriocidal proteins of serum, it adhered to cells of the host with many adhesions, which is so important for infectious process [27].

Lipase was produced from 18, 19 isolates from workers and non-workers of electrical generators respectively. While Protease was detected in 9,3 isolates of workers and non-workers of electrical generators respectively as showed at Table (5).

Researchers noticed that the lipolytic activity of lipase inactivate the immune cells by inactivating the lipoproteins of bacteria leading to be unrecognizable by macrophages [28]. Phospholipases gathered group of lipolytic enzymes which used by bacteria in order to provide a site for replication during infection. The bacterial phospholipase caused the host membranes hydrolysis, meaning destruction of host cells leading to invasion [29].

Protease enzyme had a direct role in bacterial virulence by degradation of host-associated proteins and an

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indirect role in bacterial virulence by their role in growth and proliferation, by enhancing the bacterial survival within the host environment e.g the most familiar target of bacterial protease were the host proteins which in turn involved in formation of blood clot like fibrinogen, fibrin and coagulation factors [30]. For example, the proteases of *P. aeruginosa* can directly effect on connective tissue by degrading the main component of the connective tissue, the elastin [31]. So, the proteolytic bacteria had its pounce when compared with non-proteolytic bacteria, due its direct capacity of destruction of host tissues, its tendency to be haemorrhagic and finally its role in impairing the infectious microbes clearance by defenses of host immune system [32].

The ESBLs was produced from 21,14 of workers and non-workers of electrical generators respectively as mentioned at Table (5). The multidrug-resistant bacteria of respiratory infections had been found to be so common like *E. coli*, *K. pneumoniae*, Enterobacteriaceae sp. as a result to the production of ESBL [33].

The Efflux pump was detected in 13,10 of workers and non-workers of electrical generators respectively as mentioned at Table (5). Efflux pump and biofilm are the major ways for developing the Multiple Drug Resistant (MDR). The Efflux pumps are structures composed of proteins had the ability to get rid of the different toxic materials out of microbial cells [34]. Te AcrAB efflux pump system is the responsible of the formation of MDR K. pneumoniae strains [35]. It had a crucial role in multiple antibiotic resistance e.g. Quinolones, Tetracycline, and Chloramphenicol in MDR K. pneumoniae strains [36]. Researchers found that the efflux pumps had an interesting way in both antibiotic resistant and formation of the biofilm, especially, when few studies proved a positive correlation between the antibiotic resistance of K. pneumoniae, presence of efflux pump and capacity of formation the biofilm [37]. They found 80% of biofilm-former isolates from one hundred clinical samples recorded a MDR phenotype [38].

Table (5) Virulence factors production of bacterial isolates

Virulence Factor	Workers of electrical generators No. (%)	Non-workers of electrical generators No. (%)
Capsule	20 (74.07)	18 (66.66)
Lipase	18 (66.66)	19 (70.37)
Protease	9 (33.33)	3 (11.11)
ESBLs	21(77.77)	14 (51.85)
Efflux pump	13 (48.14)	10 (37.03)

Five isolates of Klebsiella spp. Isolated from workers in generators showed high virulence, especially when length of string was (20,9,9,5 and 5) mm respectively as shown at Table (6), compared with three isolates isolated from non-workers in generators which showed high virulence, especially when length of string was (9,7 and 6) mm respectively as shown at Table (7).

Table (6) String test results of Klebsiella spp. Isolated from Generators workers

Bacterial isolate (No.)	String length (mm)	Virulence
K. pneumoniae (1 isolate)	20	High virulent

K. pneumoniae (2 isolates)	9	High virulent
K. oxytoca (2 isolates)	5	High virulent
K. pneumoniae (1 isolate)	4	weak
K. pneumoniae (4 isolate) *K. oxytoca* (1 isolate)	2	weak
K. pneumoniae (2 isolates)	1	weak

Table (7) String test results of *Klebsiella* spp. Isolated from non-workers in generators

Bacterial isolate (No.)	String length (mm)	Virulence
K. pneumoniae (1 isolate)	9	High virulent
K. oxytoca (1 isolates)	7	High virulent
K. pneumoniae (1 isolates)	6	High virulent
K. pneumoniae (2 isolate)	4	weak
K. pneumoniae (1 isolate)	3	weak
K. pneumoniae (3 isolates)	2	weak

In a study [39], 47.73% of the K. pneumoniae isolates were positive for string test, this was so close to our study.

Klebsiella spp. had the ability to gain external genetic materials which opens the door widely for extensive evolution. There are two pathotypes are causing infection, classical and hypervirulent *Klebsiella* spp., the latter had a hypermucoviscous phenotype on agar plates which could be detected by semi-quantitatively test called a "string test" [40]. The hypervirulent K. pneumoniae (hvKP) is a new and distinct variant of the *Klebsiella pneumoniae* superbug which is assumed to be an important threat globally including Asia and Western countries [41], If it compared with classical known *K. pneumoniae*, hvKP strains had improved virulence e.g. high production of capsular polysaccharides and anti-phagocytosis causing serious infections like pneumonia, pyogenic endophthalmitis, abscess of liver, osteomyelitis, and necrotizing fasciitis [42],

More than that, the presence of multi and pan drug resistant strains (PDR) strains makes controlling the infections of hvKP more interesting due to the presence of ESBLs and carbapenemases [43].

The pyogenic infections caused by hypermucoviscosity strains, which are much resistant comparing with classical *K. pneumoniae* to serum in vitro killing, neutrophils and macrophages phagocytosis, causing liver abscess and meningitis in mice [44]. Although the association between hypermucoviscosity and virulence, the nonhypermucoviscous or negative K. pneumoniae for string test may also had the genes of virulence with higher AMR [45].

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Serum resistance test showed no difference between bacterial isolates of workers and non-workers in Generators as shown at Table (8).

Table (8) Serum resistance test results for both bacterial isolates of workers and non-workers in Generator

Source of Bacterial isolates	No.(%)		
	Resistant	Intermediate	Sensitive
Workers	13	2	12
Non-workers	13	1	13

The most bacterial isolates of workers in electrical generators were weak biofilm former, it was recorded 17 isolates (62.9 %), whereas the strong isolates were only 6 isolates (22.3%), compared with non-workers who also recorded the highest no. in the weak biofilm former by 15 isolate (55.6%), whereas the strong isolates were only 5 isolates (18.5%). Statically, there were significant difference between workers and non-workers in Electrical generators at ($p<0.05$) for biofilm formation as shown at Table (9).

Table (9) Comparison between biofilm formation of isolated bacteria from workers and non- workers in Electrical generators

Groups	**Biofilm formation**	**No. (%)**	**p-value**
Workers in Electrical generators	Non	2 (7.4)	0.001*
	Weak	17 (62.9)	
	Moderate	2 (7.4)	
	Strong	6 (22.3)	
Non-workers in Electrical generators	Non	0 (0)	0.043*
	Weak	15 (55.6)	
	Moderate	7 (25.9)	
	Strong	5 (18.5)	

*p-value is significant at < 0.05 (t –tailed)

The biofilm is like a coat surrounding bacteria, protecting it from harsh environment, leading to make them more resistant to antibiotics [47]. The Antibiotics resistance correlation with biofilms is quite complex and resulted from many internal, acquired, and adaptive mechanisms [48].

The Antibiotic-resistant *K. pneumoniae*, which often produce biofilm, is characterized by high virulence due to the nature of biofilm presence [49]. Virulence factors associated with biofilm associated include production of exopolysaccharide, hypermucoviscosity, and fimbriae formation which promote the bacterial pathogenicity [50], this was noticed by presence of virulence factors in addition to biofilm.

The Hypervirulent strains of *K. pneumoniae*, including hypermucoviscous strains, also had capsule polysaccharides (CPS) for survival and immune invasion during microbial infection, which permits them to escape quickly from neutrophil-mediated intracellular killing and abscesses formation at various sites, e.g. the liver [51]. The biofilm formation had been proved to maximize virulence of *K. pneumoniae*, which leads to therapeutic challenge by becoming resistant to most classes of conventional antibiotics [52].

3. Conclusions

The study showed that microbial flora of respiratory tract of generators workers were resembling to that of

non-workers. Different levels of antibiotic resistance, in addition to different virulence factors presence (e.g. capsule, Lipase, protease and ESBLs, Efflux pump and string test) in both groups. Biofilm formation recorded a significant difference between the two groups. All this leads to conclude that generators did not effect on microbial flora of infectious pathogens of respiratory tract of the workers, which means those generators workers could be a source and reservoir for dangerous pathogenic bacteria.

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