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ORIGINAL ARTICLE

Molecular Typing of Bacterial Pathogens Isolated from Air, Non-Living Surfaces and Clinical Sources in Diyala Governorate Using MLST Technique

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ABSTRACT

The study aimed to isolate and diagnose bacterial species from clean room air samples for caesarean sections, natural childbirth, sterile preterm infants halls and the sub-corridors leading to these halls and main corridors of the hospital and from the air outside hospital building with isolation and diagnosis of bacterial species from non- living surface and clinical samples that were withdrawn from inside Al-Zahra Hospital for Women and Children in AL-Muqdadiya district in Diyala Governorate – Iraq that extends at latitudes (-3.33 and -36.6) north and longitudes (-44.22 and 45.56) east, during the period from 25 December 2021 to 19 February 2022 in light of the lack of studies that are concerned with this aspect, as 192 air samples were taken distributed to the hospital halls, such as the surgery hall and its sub-corridor leading to it, the delivery hall and its sub-corridor leading to it, the sterile preterm infant unit and its sub-corridor leading to it, the main corridor of these halls, and the air outside the hospital environment, where the air samples distributed into two groups, the first included 96 samples collected in the morning before the start of work at 8 am and the second also included 96 samples collected during Work (noon) at 12 noon ; also, 208 different swabs were collected from patients included 85 swabs after surgeries (caesarean section after suture lift after 7-10 days after delivery), 60 vaginal swabs from patients inside the delivery hall and 63 swabs from newborns lying inside the sterile preterm hall. Also, swabs were collected from non-living surfaces amounting to 100 swabs withdrawn from the natural delivery bed, caesarean section, floors, ventilation outlets, anesthesia device, fluid withdrawal device, incubators for preterm infants, ventilator and dressing cart. The total number of samples withdrawn by the effective method using the SAS air sampling device was 192, with 24 samples for each site, of which 172 samples were given a positive growth rate of 89.5%, the gram-positive bacteria constituted 69%, while the gram-negative bacteria formed an isolation rate of 31%. Sixty vaginal swabs were collected from women in the delivery hall during the examination before the birth began, 33 swabs gave a positive growth rate of 55%, of which 33 were bacterial isolated, the gram negative bacteria formed the isolates by 69.7%. 63 swabs were taken from newborns in the neonatal unit (either normal or caesarean section), those who were admitted to the preterm care hall as a result of complications during childbirth (suffering from shortness, rapid breathing and high bile in the first place, and from inflammation of the respiratory tract and urinary tract infection in the second degree), 38 swabs gave a positive growth rate of 63.3%, of which 38 bacterial isolates were mostly gram-negative bacteria. 85 swabs were taken from caesarean section wounds (7-10 days after caesarean section when the suture is lifted), 29 swabs gave a positive growth rate of 34%, the majority of which were 75.7% gram-negative bacteria. Twenty-six surface swabs gave a positive growth with a rate of 26%, of which 26 different bacterial isolates. The data of the bacterial typing analysis of the three strains of E. coli (multi-resistant bacteria) used in the molecular study by using MLST technique by using housekeeping genes showed that they are globally registered strains on the site of the Institute Pasteur in France, as the results showed that two bacterial isolates, which bear numbers (17,119) of air samples and clinical samples respectively, belong to the same strain (sequence type (ST)=741), while isolates No. (72) of surface samples belong to a different strain (ST=390). Molecular typing (MLST) was performed on three isolates of K. pneumonia (multi-resistant bacteria) where housekeeping genes were used for the purpose of testing. The strains obtained are new strains that have not been isolated before in Iraq, which means that our isolates are not registered in the MLST database at the Institute Pasteur in France.

INTRODUCTION

Hospital-acquired infections have long been identified as a serious problem affecting the quality of health care provided within health facilities, as diseases acquired through hospitals are a problem that many people around the world suffer from(1).

These diseases are the ones that are infected during health-care activities or related activities, not including diseases that the patient was infected with at the time of admission to hospital or health facility or even during the incubation period of the disease and those diseases that arise or are infected within health facilities are considered to be among the most important causes of death and these diseases which come as complications of health-care activities, cause waste of health care resources, increase cost and drain more energy(2).

Internal air quality is a great importance because it has a close correlation between in-hospital air exposure and unwanted health effects as hospitals are one of the most important internal environments responsible for the spread of airborne diseases (3).

Hospital-acquired infections are an important health problem because they have contributed to increased morbidity and mortality rates (4) and occurs in 5%-10% of all hospitals in Europe and North America and more than 40% of hospitals in parts of Asia, Latin America and Africa, and annual statistics estimate that more than 1 million people are infected (5).

The Center for Disease Control (CDC) shows the 36% of these infections can be prevented by health care worker's adherence to strict guidelines when caring for patients, the cause of the infection acquired in these hospitals is microorganism's bacteria, viruses, fungi, parasites, and these organisms may already be present on the patient's body or may come from the environment and contaminated equipment in the hospital or from health-care workers (6).

There are four main types of health care-related infections (nosocomial infections) that include (5): -

1- catheter-related urinary tract infections.
2- Pneumonia infection related to the air vent.
3- Surgical area infection.
4- Bloodstream infection

Control of the hospital air environment has received increasing attention in the light of the fact that health-care buildings have suffered from the spread of infection, particularly in developing countries with limited resources, but there are limited studies covering the relationship between natural ventilation and airborne infection, and in general the purpose of ventilation any place busy with users is to provide fresh air to occupants and remove the heat generated in the place (7) .

Two types of bacteria have been typed using the MLST technique (Multi locus sequence typing) is a relatively recent molecular biological technique. Widely used for molecular typing.

There are numerous examples of their use to describe the demographic composition of pathogens, study vaccines, track the transmission of epidemic strains, and identify disease-related malignant species and strains (8).

Due to the increase in the nosocomial infections and the patients suffering from delayed recovery after surgical interventions in addition to the lack studies in Diyala province in this aspect and due to the clinical importance of isolated pathogens, the study was carried out at the level of hospitals in al-Muqdadiya- district, which aims to isolation of microbial types of clean room air and sub-corridors leading to rooms and main corridors, in addition to isolating microbial types of atmospheric air outside the hospital building, as well as isolating microbial types from clinical samples that were withdrawn from patients hospitalized and molecular analysis of the results using MLST technology to find out the source of the pathogenic strains of hospital-acquired infections.

MATERIALS AND METHOD

Samples Culture: The samples (wound swabs, patients swabs, contamination control swabs) were planted immediately on the blood agar, MaCconkey agar, and solid mannitol medium, and all dishes were incubated at (37 ° C) for a period of (24 hours), after which a number of diagnostic, phenotypic and biochemical tests of bacteria were performed.

Diagnosis of bacterial isolates

First: Phenotypic diagnosis: The phenotypic characteristics of the growing colonies were used on the medium of blood agar, MaCconkey agar and mannitol agar, in terms of the forms of apparent colonies, texture, color, and size, as well as the observation of other general characteristics such as lactose fermentation or not, as well as the presence of hemolysis on the medium of blood agar or not in the initial diagnosis of bacterial isolates.

Second: Microscopic characteristics: Microscopic examination of bacterial cells in colonies was performed under the oily lens of the light microscope after dyeing them with a gram pigments and determining their interaction with the dye and studying the shapes of the cells and their clusters.

Third: Biochemical tests: Biochemical tests were carried out according to the manufacturer's instructions for the purpose of diagnosing bacteria.

Klebsiella pneumonia Typing with MLST

Primers of Klebsiella pneumonia: K. pneumonia isolates isolated from air, non-living surfaces and patients were typed with MLST using seven housekeeping genes and identified the nucleotide sequence at specific sites. The genes used are (rpoB, gapA, mdh, pgi, phoE, infB, tonB) and the primers shown in Table 1 were used to amplify the segments identified in the genes used in typing.

Escherichia coli Typing with MLST: Isolates of E. coli isolated from air, non-living surfaces and patients were typed with MLST using eight housekeeping genes and the nucleotides sequence was identified at specific sites. Genes (pabB2, polB2, putP, trpA, trpB, uidA, dinB, icdA) The primers shown in Table 2 were used to amplify the specific segments in the genes used in typing.

Table 1: primers of k.pneumonia

Primer Name	Sequence 5`-3`	Annealing Temp. (ºC)	Product size (bp)
rpoB-F	GGCGAAATGGCWGAGAACCA	55	1075
RpoB-R	GAGTCTTCGAAGTTGTAACC		
GapA-F	TGAAATATGACTCCACTCACGG	55	662
GapA-R	CTTCAGAAGCGGCTTTGATGGCTT		
Mdh-F	CCCAACTCGCTTCAGGTTCAG	55	756
Mdh-R	CCGTTTTTCCCCAGCAGCAG		
Pgi-F	GAGAAAAACCTGCCTGTACTGCTGGC	55	566
Pgi-R	CGCGCCACGCTTTATAGCGGTTAAT		
PhoE-F	ACCTACCGCAACACCGACTTCTTCGG	58	602
PhoE-R	TGATCAGAACTGGTAGGTGAT		
InfB-F	CTCGCTGCTGGACTATATTCG	55	462
InfB-R	CGCTTTCAGCTCAAGAACTTC		
TonB-F	CTTTATACCTCGGTACATCAGGTT	55	539
TonB-R	ATTCGCCGGCTGRGCRGAGAG		

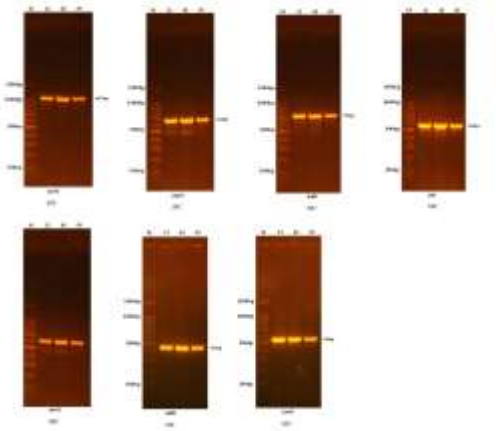

Figure 1: Results of the presence of 7 MLST genes of Klebsiella pneumonia strains were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. Lane1:100bp DNA marker.

Table 2: Primers of Escherichia coli

Primer Name	Sequence 5`-3`	Annealing Temp. (ºC)	Product size (bp)
pabB2oF	AATCCAATATGACCCGCGAG	55	598
pabBoR	GGTTCCAGTTCGTCGATAAT		
polB2oF	GGCGGCTATGTGATGGATTC		615
polBoR	GGTTGGCATCAGAAAACGGC		
putP2oF	CTGTTTAACCCGTGGATTGC		506
putPoR	GCATCGGCCTCGGCAAAGCG		
trpAoF	GCTACGAATCTCTGTTTGCC		784
trpAoR	GCTTTCATCGGTTGTACAAA		
trpB2oF	CACTATATGCTGGGCACCGC		632
trpBoR	CCTCGTGCTTTCAAAATATC		
uidAoF	CATTACGGCAAAGTGTGGGTCAAT		658
uidAoR	CCATCAGCACGTTATCGAATCCTT		
dinB-F	TGAGAGGTGAGCAATGCGTA		606
dinB-r	CGTAGCCCCATCGCTTCCAG		
icdA-F	ATTCGCTTCCCGGAACATTG		677
IcdA-R	ATGATCGCGTCACCAAAYTC		

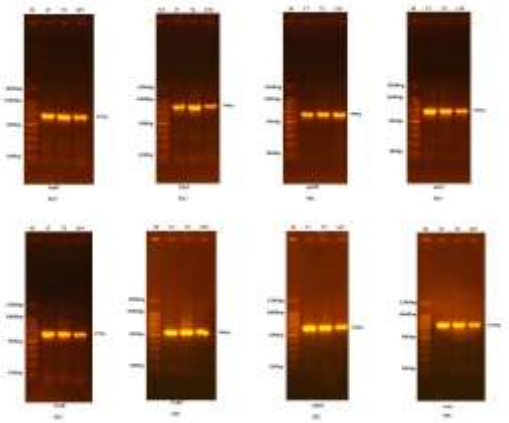

Figure 2: Results of the presence of 8 MLST genes of Escherichia coli strains were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. Lane1:100bp DNA marker.

Identify nucleotide sequences in duplicated gene fragments: PCR amplification products were sent to all strains to sequence nucleotides with Sanger technology using the ABI3730XL automated DNA sequencing device, by Macrogen Corporation - in Korea, all locations on both strands were sequenced. The data was stored and analyzed using Genious Prime 2019 (https://www.geneious.com) software. For each gene, the characteristic alleles were identified and numbered using the PubMLST (https://bigsdb.pasteur.fr) site. and select sequence type (ST) using the same database.

RESULTS AND DISCUSSION

Bacterial species isolated from hospital environment samples through the current study, we notice that there is a variation in the isolated percentages of microorganisms (negative and positive) because the spread depends on the nature and livelihood of the bacteria as in Table 3

Table 3: Numbers, types and percentages of bacteria isolated from samples Hospital environment

Bacteria	Gram stain				Total	
	Positive		Negative			
	%	NO.	%	NO.	%	No.
Staph. Aureus	56	80	0	0	26.85	80
Staph . epidermidis	44	62	0	0	20.81	62
K.pneumonia	0	0	23	36	12.08	36
K.aerogens	0	0	36	56	18.79	56
P.aerogens	0	0	12	18	6.04	18
E.coli	0	0	27	42	14.09	42
Proteus	0	0	3	4	1.34	4
Total	100	142	100	156	100	298

The great variation of bacterial species obtained during the study of the sites from which samples were drawn, whether air samples, surfaces or clinical, reflects a major problem suffered by health institutions, which is the problem of controlling pollution within them, especially within sensitive sections such as surgical rooms, premature and childbirth halls, in addition to burn halls and intensive care halls, as these places (environments) are supposed to be sterile and isolated from the external environment to ensure a kind of stability for the species. Bacterial inside, as many acute diseases and allergies are caused by the presence of a large number of microorganisms, which reflects the cleanliness and sterilization of the internal environment of the halls, which are usually occupied by people (9).

The results showed that S. aureus and S.epidermis bacteria occupied the lead in the isolation rate by 26.85% (80 isolates) and 20.81% (62 isolates) respectively, and these results study are consistent with what came when isolating environmental and clinical samples from AL-Diwaniyah Teaching Hospital(10), and explains the reason for the spread of these bacterial species to their resistance to antibiotics, disinfectants and sterilizers used in hospitals, where the flow resistance mechanism is one of the most common mechanisms in staphylococcal resistance, which indicates the need to monitor Staphylococcus isolated from hospitals according to the recommendations of the British system, as these bacteria and their types (golden and epidermdis) are one of the causes of pollution and injuries in hospitals (11).

Followed in the percentage of isolation bacteria K. aerogens by 18.79% (56 isolation) as it can live between the folds of the skin as well as its ability to stay on surfaces and hands for more than 20 minutes being resistant to sterilizers and disinfectants significantly and can cause severe injury to those lying in hospitals, especially premature babies and intensive care departments for childbirth, followed by the percentage of isolation of E. coli by 14.09% (42 isolation), where it exists as a natural inhabitant in the intestines of humans and animals, and sometimes turns to pathogenic bacteria (12), followed by K.pneumoniae isolation at 12.08% (36 isolates),as its infections are often opportunistic in people with chest diseases, diabetics and malnutrition patients, and thus it plays an important role in causing nosocomial infections (13), followed by the isolation rate of P. aeruginosa bacteria by 6.04% (18 isolates), where P. aeruginosa bacteria are characterized by their ability to colonize non-living surfaces in hospitals and cause nosocomial infections and their ability to invade the body through medical instruments placed in patients (14), and finally the bacterium of proteus by 1.34% (4 isolates), which is the main causative agent of acquired diseases in up to (90%) of intensive care corridors in hospitals, especially from patients with recurrent injuries and people with urinary tract diseases (15).

The results of a study conducted on the floors of Ramadi General Hospital showed that the levels of microbial contamination in the samples isolated from sources including air conditioning pipes, beds of inpatient patients, hospital floor, as the percentages were different, as the percentage of bacterial contamination in the internal surfaces of the air conditioning pipes was the highest among the rates of bacterial contamination and for all sites by 38.3%, followed by the hospital floor by 20.1% and finally the beds of inpatient patients by 15.7%, meaning that the air conditioning pipes were not paid attention to at all and were not provided with filters or sterilized by modern methods such as ultraviolet rays and others, which led to a very high probability that these tubes are the main focus of pollution inside the hospital and that the air passing through them is what transports the various pathogens to the beds and floor of the hospital and to the bodies of the people present, whether patients or workers, which leads to their adhesion to the wounds and thus inflammation or even inhalation by the sleeping patients, which leads to the emergence of respiratory problems, systemic diseases, various allergies and health problems. In general, especially in unvaccinated patients, our study also agreed with the results of the same study mentioned above that most bacterial species isolated from non-living surfaces were gram negative bacteria (16).

Isolated types of pollution control swabs : The total number of surface swabs (control of contamination) was 100 swabs withdrawn from the natural delivery bed, caesarean section, floors, ventilation outlets, anesthesia device, fluid withdrawal device, incubators for preterm infants, ventilator and dressing cart.

Twenty-six swabs gave a positive growth with a germination rate of 26%, of which 26 different bacterial isolates, including 10 isolates of E. coli and K. aerogens bacteria (10 isolates), each of which constituted 38.5% of the isolates, followed by P. aeruginosa (4 isolates) with 15.4%, and K. pneumonia came with the lowest isolation rate of 7.7% of isolates (2 isolates), as shown in Table 4.

Table 4: Number and percentages from pollution control swabs

Bacteria	count	%
E.coli	10	38.5
K.pneumonia	2	7,7
K.aerogens	10	38.5
Pseudomonas	4	15.4
Total	26	100

Isolated types from clinical samples

Vaginal swabs at birth: 60 vaginal swabs were collected from women in the delivery hall during the examination before the birth began. 33 swabs gave a positive growth rate of 55%, of which 33 were isolated, the negative bacteria of gram isolates constituted 69.7%, the highest isolation rate was for K. aerogens by 27.3%, followed by E. coli 24.2%, followed by K. pneumonia bacteria with an isolation rate of 18.2%, followed by Staphylococcus bacteria with an isolation rate of 15.2%, P.aeruginosa bacteria with an isolation rate of 12.1%, and finally S.epidermids bacteria with the lowest isolation rate of 3.0%.

Baby swabs in the neonatal unit: Sixty-three swabs were taken from newborns admitted to the neonatal unit (normal or caesarean section), who were admitted to the preterm care room due to complications during childbirth (suffering from shortness, rapid breathing and high bile in the first place, and from respiratory tract infection and urinary tract infection in the second degree),38 swabs gave a positive growth rate of 63.3%, of which 38 bacterial isolates were mostly gram-negative bacteria. The highest isolation rate was for K. pneumonia 34.2%, P. aeruginosa 18.4%, while K. aerogens and S.aureus recorded equal isolation rates of 15.8%, and E. coli and S. epidermids recorded equal percentages of 7.9%.

Wound swabs after cesarean sections: 85 swabs were taken from caesarean section wounds (7-10 days after caesarean section when the suture is lifted). 29 swabs gave a positive growth rate of 34%. Of these, 29 isolates were distributed among the bacterial species K. pneumonia and aerogens with the highest isolation rates of 24.1% each, followed by E.coli bacteria 17.2%, S.epidermids bacteria 13.8%, and the lowest isolation rate was for P. aeruginosa bacteria by 10.3%, as shown in table 5:

Table 5: Types and Percentages of Bacterial Isolates from Clinical Samples

Bacteria	Swabs						Total	
	Wound		Noenatal care		Vaginal			
	%	N0.	%	N0.	%	N0.	%	No.
S.aureus	10.3	3	15.8	6	15.2	5	14.0%	14
S.epidermids	13.8	4	7.9	3	30.0	1	8.0%	8
K.pneumonia	24.1	7	34.2	13	18.2	6	26.0%	26
K.aerogens	24.1	7	15.8	6	27.3	9	22.0%	22
Pseudomonas	10.3	3	18.4	7	12.1	4	14.0%	14
E.coli	17.2	5	7.9	3	24.2	8	16.0%	16
Total	100%	29	100%	38	100%	33	100%	100

The control of infections that occur in wounds after surgeries is strongly affected by the quality of cleanliness and sterilization of operating rooms, which is determined by the structural features of clean halls, their systems and the behavior of their workers, as the main sources of pollution, especially in clean surgeries, are the patient's skin, airborne particles from workers in operating rooms, where a study conducted by the British Research Council showed a relationship between microbial air pollution and the occurrence of surgical site infections (17).

It is associated with the emergence of strains resistant to sterilizers, detergents and antibiotics and the increase in surgical patients who suffer from chronic diseases that weaken the immune system, and in order to reduce and control the risk of this infection, effective and organized control methods must be followed and applied with full awareness and knowledge that this risk is affected by several factors related to the patient, the surgery, hospital staff and the hospital itself (18).

A study conducted in Italy/Milan hospitals showed that when performing general surgeries such as laparoscopic gallbladder lift, inguinal hernia, mastectomy, ENT surgery, urology, neurosurgery, and orthopedics, the main non-compliance was room congestion, mainly due to the entry of non-surgical staff where the number of people present in the operating room at the same time was from 6 to 12 (average 9) and in all cases there was a traffic flow due to people who did not in relation to the operation, also the incorrect performance of skin sterilization before surgery, which in seven out of 10 operations was carried out without waiting for the automatic drying of the disinfectant (19), and almost all these violations of the protocol are present in our hospitals.

Molecular typing : Typing was done using multi locus sequence typing (MLST) technique using housekeeping genes that showed the type of sequence obtained for Escherichia coli isolates, where the technique used showed that the type of sequence obtained for clinical isolation No. (17) is the same as for air isolation No. (119), which supports that the pollution inside the hospital (hospital infections) is the result of atmospheric air pollution.

The results of the resistance test showed that the clinical isolation No. (17) was resistant to the antibiotics (amoxiciline - claviolinic acid, ceftriaxone, cefachlor, pipracillin, naldixic acid, norfloxacin) while the air isolation No. (119) was resistant to the antibiotics (amoxicline - claviolinic acid, ceftriaxone, cefachlor, pipracillin, naldixic acid, norfloxacin, aztreonam) As for the environmental isolation from surfaces No. (72), it was resistant to the antibiotics (amoxiciline - claviolinic acid, ceftriaxone, cefachlor, pipracillin, aztreonam), from these results, we observe that aerobic isolation was more resistant to antibiotics.

As for the bacteria K. pneumoniae, the three isolates were new isolates (New strains), that is, ST is not registered in the Pasteur Institute, and the results of the resistance showed that the isolates were resistant to the following antibiotics; Air isolation No. (21) was resistant to the antibiotics(amoxcilin-claviolinic acid, cefachlor, nitrofurine, ceftriaxone, aztreonam, piperacillin, imepenem, streptomycin, azthromycin, naldixic acid), environmental isolation from surfaces No. (45) was resistant to antibiotics (amoxciline-claviolinic acid, cefachlor, nitrofurine),Clinical isolation No. (59) was resistant to antibiotics (amoxcilin-claviolinic acid, cefachlor, nitrofurine, ceftriaxone, aztreonam, pipracillin), we note here also that aerobic isolation was also the most resistant isolates to the antibiotics used, as 10 out of 12 antibiotics used in the examination resisted. As shown in Table 6.

Multiple resistance to antimicrobials is usually associated with the proliferation of transmissible plasmids or by the acquisition of resistance genes that occur through horizontal transfer of genes, and some reports indicate that bacteria have many virulence factors that contribute significantly to the pathogenicity and resistance of bacteria such as adhesion agents, capsule and polysaccharides (20).

Table 6: Results of Resistance Test for Bacterial Isolates Used in MLST Molecular Profiling

Antibiotic for sensitivity test	No. of sensitive isolates	No. of resist isolates
Pipracillin		K21, K59,E17, E72, E119
Amoxicillin / Clavulanic acid		K45, K59 ,K21, E17, E72, E119
Cefachlor		K45, K21, K59, E17, E72, E119
Ceftriaxon		K21, K59, E17, E72, E119
Aztreonam		K21,K59, E72,E119
Impenem	K45, K49, E17, E72, E119	K21,
Streptomycin	K59, E17, E72, E119	K21
Gentamicin	K21, K45, K59, E17, E72, E119	
Azithromicin	K45, K49, E17, E119, E72	K21
Nitrofuration	E17	K45,K21
Nalidixic acid	K45, K49	K21, K59, E17 , E119
Norfloxacin	K21, K45, K59, E72	E17, E119

(E)=E.coli / (K)= K.pneumonia

Genetic characterization of pathogens has become an important target in the detection of infectious or pathogenic agents and genotyping tools can be used in the detection of pathogens and characterization of pathogenic organisms (21). In the present study, two types of bacteria were selected and profiled using three isolates for each type as shown below.

K. pneumoniae is an opportunistic bacterial pathogen that usually causes urinary tract infection, pneumonia, inflammation of wounds and burns, septicemia, meningites (22). In recent years, Klebsiella has become an important cause of hospital infections, as antibiotics are widely used for the purpose of their treatment as they are widely antibiotic-resistant bacteria, which is a major public health concern worldwide (21). Multi-locus sequence typing were used to typing K. pneumoniae and this technique characterized microbial species using DNA sequences of the inner part of housekeeping genes (23). The aim of using the technique was to find out the types of strains found in environment (Non-living surface and air isolates) and clinical isolates.

Molecular typing was performed using (MLST) technology for three isolates of K. pneumonia multi-resistance, where housekeeping genes were used for the purpose of conducting the test, namely (ropB, gapA, mdh, pgi, phoE, infB, tonB.) where the PCR reaction was conducted according to the sequence of primers of the genes and according to what was mentioned in the chapter of materials and methods of action according to the official website of the Pasteur Center / France.

STs were classified on the basis of molecular sequencing results of seven of the housekeeping genes, and for the purpose of detecting the allele heterogeneity of the seven housekeeping genes, the PCR reaction products were sent to Macrogen in South

Korea for the purpose of sequencing nitrogenous bases, where the results were obtained and analyzed using Genius prime/2019 software (https://www. genius .com) and the strains in the current study were identified with the database that have a minimum similarity in profile Based on the matrix of pairwise differences between the allele profiles of the strains, our results showed that the strains obtained are new strains that have not been isolated before in Iraq, which means that our isolates are not registered in the MLST database at the Pasteur Institute in France. The results of the current study are consistent with those of other studies that conducted molecular typing of Klebsiella bacteria (MLST) isolated from different sources (24).

Table 7: Alleles Housekeeping Genes for K. pneumoniae

Bacteria / Sample	Klebsiella pneumonia		Housekeeping gene allelic profile							ST
	N0.isolate	Detailes sample	tonB	RpoB	PhoE	Pgi	Mdh	infB	GapA	
Clinical sample	59	Mouth swab from baby sleeping in neonatal care unit	24	195	13	1	1	6	1	NEW STRAIN
Air sample	21	Air sample from sub- corridor leading to neonatal care units	77	287	26	1	1	6	2	
Surface sample	45	Inubator in neonatal care units	24	195	1	1	1	6	1	

The numbers in Table 7 under each gene refer to the number of the gene registered in the Pasteur Institute after comparing them with the housekeeping genes of Klebsiella pneumonia under study, while the code (ST) refers to Strain typing, which means the type of strain, as each strain has a special code registered in the Pasteur database, and the table above shows that the three strains that have been new strains.

E.coli is a type of Enterobacterieaciae and is naturally found in multiple areas of the human body, it is an opportunistic pathogen that infects the body wherever the opportunity arises, causing septic wound and meningitis in children, and infecting the urinary system (25).

Multi locus sequencing typing (MLST) writing technique was used to characterize E. coli isolates among other bacterial species based on the sequencing difference of eight sites in the housekeeping genes (pabB2, polB2, putP, trpA, trpB, uidA, dinB, icdA) as in Table 8 where different allele numbers are assigned to different nucleotide sequences for each position, and then each strain is identified by the alleles of the seven sites (allele profile), closely related organisms can be grouped into germinal complexes, Analyses of E. coli isolates by MLST help to determine the genetic relationship of isolates, which is important for molecular epidemiological and evolutionary studies (26).

Molecular profiling was performed using (MLST) technology for three isolates of multi resistant Escherichia coli, where housekeeping genes were used for the purpose of conducting the test, namely (pabB2, polB2, putP, trpA, trpB, uidA, dinB, icdA), as the PCR reaction was conducted according to the sequence of primers of genes and according to what was mentioned in the separation of materials and methods of work according to the official website of the Pasteur Center in France, where the products of the PCR reaction were sent to Macrogen in South Korea for the purpose of determining the sequence of nitrogenous bases, where on results and analysis using genius prime/2019 software (https://www. genius .com)

Table 8: Alleles Housekeeping Genes for Escherichia coli

Bacteria / Sample	E.Coli		Housekeeping gene allelic profile								ST
	N0.isolate	Detailes sample	DinB	icdA	pabB	polB	PutP	trpA	TrpB	UidA	
Clinical sample	17	Vaginal swab for a patient inside the delivery hall	8	262	7	3	7	1	4	2	741
Surface sample	72	Fluid intake device in the delivery hall	8	2	7	3	131	1	4	2	390
Air sample	119	Air sample from the sub-corridor outside the delivery hall leading to it	8	262	7	3	7	1	4	2	741

The data of the bacterial profiling analysis of the three strains of E. coli used in the molecular study showed that they are globally registered strains on the site of the Institute Pasteur in France, as the results showed that two bacterial isolates, which bear numbers (17,119) of air samples and clinical samples respectively, belong to the same strain (ST=741), while isolates No. (72) of surface samples belong to a different strain (ST=390). From here it is clear that the isolated E. coli strains in our study may be sourced to the world from Iraq or vice versa as a result of travel and movement between countries or import and export of various goods that increase their spread.

CONCLUSIONS

1- The most isolated bacteria were Cram-positive bacteria, including Staphylococcus aureus and Staphylococcus epidermis, while the gram-negative bacteria were the isolated types of Klebsiella pneumonia, aerogens, E. coli, Pseudomonas aeruginosa and proteus bacteria.
2- The emergence of severe and multiple resistance against most of the antibiotics used in the examination of sensitivity to isolated bacterial species.
3- Attention to ventilation systems and maintenance according to a specific schedule during the year has a major role in reducing pollution inside the halls.
4- The results of the molecular typing of the study showed a relationship between hospital infections and bacterial pollution of the air inside the clean halls.

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