
Evaluation and Susceptibility Pattern of Airborne Gram Negative Bacilli Bacteria in Operating Theaters and Intensive Care Units in North Jordan Hospitals, Jordan

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Abstract:

The objective of this study is to evaluate the presence of airborne Gram Negative Bacilli in the operating theatres (OT), intensive care units (ICU) and nursery intensive care units (NICU) of north Jordanian hospitals, and their susceptibility to several antibiotics. 244 air samples of 100 liters volume/min were collected by a microbiological air sampler from the above units during the period of seven months. Air samples were impacted on trypticase soy agar (TSA) then incubated at 37 °C for 48 hours. Each bacterial colony that subsequently appeared on agar plates were sub-cultured on blood agar and MacConkey Agar with incubation at 37 °C for 24-48 hours, and then identified by standard methods. The average bacterial count in the OT, ICU and NICU ranged between 0 to 1200, 30 to 1160, 40 to 1130 CFU/m³, respectively. Gram Negative Bacilli comprised 2.35%, 1.57% and 1.58% in OT, ICU and NICU, respectively. Of all Gram Negative Bacilli *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Acinetobacter* spp. comprised of 42%, 18.7% and 17.4% respectively in all studied units. However, *Aeromonas* spp. comprised of 10% isolated only in ICU and NICU. In addition *Enterobacter cloacae*, *E. sakazakii* and *Proteus* spp. comprised of 3.4%, 6.3% and 1.5% respectively being present in the OT only. Gram Negative Bacilli showed a considerable resistance to some antibiotics, the highest for Ceftriaxone 25.1%, followed by Nalidixic acid with 24.6. Cefuroxime and Cefaclor resistance was 22.6%. The lowest resistance rate was observed with Gentamycin 9.4%, Ciprofloxacin 10%, Imipenem 10.4% and Amikacin 11.4%.

Key Words: Airborne, Antibiotic, Bacteria, Gram negative bacilli, Hospital

Introduction:

Airborne microorganisms are present in all indoor environments; most of them in the indoor air originate from humans.^{1,2} The presence of high concentrations of airborne microorganisms singles a poor air, which is associated with human health effects, including allergic and irritant responses, infectious diseases, respiratory problems, and hypersensitivity reaction.^{3,4}

The hospital indoor environment may contain a diverse range of microbial population that includes several pathogenic and opportunistic bacteria, viruses and fungi.⁵ Shekhawat *et al.* (1992) stated that the hospital environment is full of pathogens that may influence the health of both patients and hospital staff and may cause nosocomial infections (hospital-associated infections, HAI). The hospital environment consists of many components, which have a direct bearing upon HAI including design of wards, operating theater facilities, air quality, water supply, food and handling of medical waste and laundry.⁶

Contaminated air droplets from patients, staff carriers³ and outdoor air are possible sources. The number of organisms depend on the number of people occupying the room, the amount of activity, rate of air exchange, number of visitors and the amount of materials brought in from outside are recognized as sources of hospital contamination.⁷

The programmed monitoring of the quantity and types of airborne microorganisms is important since these values can be used as an index for the cleanliness of the environment and source of hospital-acquired infections.^{8,9}

Environmental and Human related organisms (particularly the Gram Positive Cocci and Gram Negative Bacilli⁶ can distributed in large areas of hospitals through different mechanisms.^{10,11} These are significantly associated with more morbidity and mortality, and increasing the cost of prevention and patients hospitalization period.^{5,7}

The number of micro-organisms in some sensitive hospital areas such as OT, ICU and

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NICU is usually low because the high sanitary standards applied compared to other hospital areas.^{6,12}

The closed environment, type of microorganisms and antibiotics used in these units may provide suitable conditions for the spread of infectious agents. In different studies researchers indicated that the environment biocontamination of these hospital units are associated with surgical infections, neonatal sepsis and nosocomial infection.¹²⁻¹⁴

Many types of Gram Negative Bacilli such as *Klebsiella*, *Pseudomonas aeruginosa*, *Citrobacter*, *Proteus*, *Escherichia coli*, *Acinetobacter* and *Enterococcus* were found to contaminate the environment of ICUs.¹⁵

Shekhawat et al. (1997) reported many types of bacteria in large number when carried in the air of pediatric wards and neonatal nursery.⁸ *Chandrashekar et al.*¹⁸ found that the indoor air of NICU carries common nosocomial bacteria of four or more different bacterial species at dangerous levels.^{16,17} Therefore, the HAI in Pediatric and NICUs represent an important cause of morbidity and mortality in these populations.¹⁸

In addition to pathogens, several opportunistic bacteria, such as *Pseudomonas aeruginosa*, *Klebsiella*, *Proteus*, *Citrobacter*, *Enterobacter* and *Acinetobacter* may be able to infect immuno-suppressed patients. These bacteria may be isolated and transmitted via the air of hospitals.^{15,18,19} *Acinetobacter* spp. can be isolated from soil, sewage, water and human skin. The drug resistant strains of *Acinetobacter* are becoming more frequent, whilst the infection caused by these strains are often difficult to treat.^{16,20}

P. aeruginosa is a significant human pathogen that usually causes infections only in the context of some serious underlying disease. Transmission of *P. aeruginosa* from patient to patient can become airborne during certain procedures like removal of dressings from leg ulcers or puncturing of an abscess.²¹ *P. aeruginosa* can be difficult to eradicate from hospital wards, as it is resistant to, and may multiply in, many of the disinfectants and antiseptics commonly are used in hospitals.²² Some other Gram Negative Bacteria may also cause nosocomial infection through the air, although this is not a major route of transmission due to their poor survival in dry conditions.²³

The number and diversity of air borne contamination in the operating rooms are varieties significantly between times of

sampling, and the rate of post-operative wound infection,¹⁴ and highly affected by ventilating system¹⁶ and internal organization and design of hospital building itself.²³

Materials and methods:

The studied hospitals

The study was carried out in north of Jordan over three departments of six different hospitals serving a population of about 1.3 million. Airborne Gram Negative Bacteria were studied in operating theatres, intensive care units of the hospitals.

Collection of air samples

During the period between June and December /2005, a total of 244 air samples were collected from three sensitive units. Each air sample was collected by a microbiological air sampler (M.A.Q.S.II-90 / OXOID, UK) that holds 90 mm Petri dishes within an autoclavable anodized aluminum head of 380 holes. The sampler set at an air-sampling rate 100 L/min for two minutes per sample. Duplicated air samples were collected during each visit at different sites of each unit. They were collected at one meter from the floor (approximating the level of the patient's bed). Samples from the operating theatres were collected before the operation when the room was empty.

Sample processing

After impacting the air samples on Tryptic Soy Agar (TSA) for total bacterial count, and MacConkey Agar (Oxoid, UK) for isolation Gram Negative Bacteria, they were transported to the laboratory and immediately incubated at 37 °C for 48 h. Diversity and total counts of bacteria on TSA plates were recorded by using the colony counter (560, Suntlet, Labolan).

Isolation and identification of bacteria

Each Gram Negative Bacilli colonies grow in MacConkey Agar was sub-cultured individually in another MacConkey Agar plates for isolation the bacteria in a pure form and for further identified agar plates incubated aerobically at 35 °C for 24-48 hours. Growth of bacterial colonies evaluated according to standard methods.²⁴

Identification of Gram Negative Bacilli based on the morphological characterization of each isolate was first performed, including color, size, and colony characteristics (form, margin, and elevation). The Gram stain test was performed on each isolate. Members of the family *Enterobacteriaceae* and other non-fastidious, non-enteric Gram Negative Bacilli such as *Pseudomonas*, *Acinetobacter*, *Aeromonas* were further identified using the

API 20 (E or NE) system (Biomerix, France). The test was performed according to the manufacturer instruction. Strips were incubated aerobically at 37 °C for 24 hours and the results were interpreted by change in the color and the use of analytical profile index (Biomerix, France, Ref. 20 190). All results were collected on the provider data sheet, compared and confirmed with the results obtained from the conventional identification tests and methods such as Gram stain and conventional biochemical tests.²⁵

Antimicrobial susceptibility test

Plates and inoculums preparation

Test organism from isolated culture was activated by inoculation into Mueller-Hinton broth and/or trypticase soy broth (Oxoid, UK) then incubated at 37 °C for 24 hours. The

turbidity of organisms in the broth was adjusted to be equal to or greater than 0.5 McFarland turbidity standards (1.5×10^8 CFU/ml). Mueller-Hinton agar plates and 0.5 McFarland standards were prepared according to the recommendation of Kirby-Bauer disc diffusion procedure (26) and NCCLS (27). The test organisms were homogeneously inoculated by a sterile cotton swab on the surface of two freshly prepared Mueller-Hinton agar plates. Plates were incubated at 37 °C for 24 hours.

Application of disks and incubation

Twenty two different antibiotics discs (Oxoid, UK) (Table 3) were used for susceptibility test of Gram Negative Bacilli. Antibiotic disks were applied to the agar surface by a sterile forceps and then gently pressed to assure complete contact of disks on the agar surface.

Table 3: Antibiotics panel used for Gram Negative Bacilli and their percentage of sensitivity.

Antibiotics	Symbol	%	<i>P. aeruginosa</i>	<i>E. cloacae</i>	<i>E. sakazakii</i>	<i>proteus spp</i>	<i>C. freundii</i>	<i>Acinetobacter</i>	<i>Aeromonas</i>	<i>Total</i>
			42.8	3.4	6.3	1.5	18.7	17.4	9.9	100
		Concentration (µg)	Sensitivity							
Amikacin	AK	3	85	88	90	100	100	74	83	88.6
Amoxicillin	AX	25	62	71	86	93	98	82	85	82.4
Ampicillin	AM	10	70	66	91	96	92	86	75	82.3
Cefaclor	CEC	30	60	68	86	88	86	90	64	77.4
Cefotaxime	CTX	30	66	88	89	92	88	74	68	80.7
Chloramphenicol	C	30	82	90	83	95	90	83	79	86
Ciprofloxacin	CIP	5	86	93	88	96	92	89	86	90
Cloxacillin	CX	30	73	65	82	89	82	86	87	80.6
Erythromycin	E	15	77	78	83	86	81	83	80	81.1
Imipenem	IPM	10	92	85	89	100	100	81	80	89.6
Nalidixic Acid	NA	20	60	71	80	85	86	75	71	75.4
Penicillin	P	10	72	73	75	82	83	80	75	77.1
Rifampin	RA	10	75	77	83	88	81	78	76	79.7
Trimethoprim	SXT	30	77	82	86	84	88	90	80	83.9
Ceftriaxone	CRO	30	55	71	79	80	86	83	70	74.9
Cefuroxime	CXM	30	72	74	82	86	79	76	73	77.4
Tetracycline	TE	30	75	87	88	90	76	83	77	82.3
Augmentin	AUG	30	73	81	83	81	88	91	92	84.1
Linezolid	LZD	30	71	80	86	88	76	79	73	79
Rifampicin	RP	5	81	86	89	94	73	86	80	84.1
Gentamicin	GN	120	93	87	86	96	100	84	88	90.6
Streptomycin	S	300	74	82	78	85	89	79	77	80.6

Plates were then inverted and incubated at 35°C for 16-18 hours. The results of susceptibility test (inhibition zone diameter) for each isolate were measured in millimeter and interpreted according to NCCLS standards on a special data sheet.

Statistical analysis:

Data were processed by the SPSS statistical software (SPSS for windows, Version 10.0.1, SPSS Inc, USA). Comparisons between groups (Hospitals and Units) were tested by one way ANOVA test and Comparison between two independent samples were done through the use of the T-test. Two tailed P values < 0.05 was considered significant.

Results and discussion:

Number and diversity of isolated bacteria

Table 1: Number and Diversity of isolated bacteria/m³ in operating theatres (OT), intensive care units (ICU) and nursery intensive care units (NICU) of north jordanian Hospitals, Jordan.

Bacteria (Number, Diversity)	Operating Theatres (OT)	Intensive Care Units (ICU)	Nursery Intensive Care Units (NICU)
Bacterial Count			
Range	0-1200	30-1160	40-1130
Average	218	312	322
Bacterial Diversity			
Range	0-32	2--23	2--25
Average	10	11	12

Diversity of bacteria usually related to the count. In this study, the observed bacterial diversity was similar in all hospital units (P-value = 0.007). Ross & Menezes (2004) suggested that the high microbial biodiversity is associated with high temperature and relative air humidity that favors microbial

In this study the bacterial count in OT, ICU and NICU ranged between 0 to 1200, 30 to 1160 and 40 to 1130 CFU/m³, respectively. Diversity of bacteria in OT, ICU and NICU ranged between 0 and 32, 2 and 23, 2 and 25, respectively (Table 1). Total air bacterial count and diversity in all units were significantly different (P-value = 0.007). Bacterial counts in OT were less than ICU, NICU which consistent with another study.^{11,28} This could be due to the high sanitary standards in OT, as compared to other hospital areas. These finding could possibly be explained by other factors such as the number of visitors, cases of patients, opened doors and the amount of material brought from outside, such as flowers and fruits.^{3,21} These recognized as sources of hospital contamination.⁵

growth. Beggs (2003) reported that approximately two thirds of microorganisms isolated from the hospitals air were Gram Positive Cocci, with higher proportion of microorganisms in operating theaters. The results of this study, as detailed in (Table 2) found similar results.

Table 2: Percentage distribution of Gram Negative Bacteria in operating theatres (OT), intensive care units (ICU) and nursery intensive care units (NICU) of north jordanian Hospitals, Jordan.

Percentage and distribution of isolated Gram negative bacteria/ m ³	Operating Theatres (OT)	Intensive Care Units (ICU)	Nursery Intensive Care Units (NICU)	Total %
	2.4	1.57	1.58	5.5
<i>Pseudomonas aeruginosa</i>	37	46.4	45	42.8
<i>Enterobacter cloacae</i>	10.2	ND	ND	3.4
<i>E. sakazakii</i>	19	ND	ND	6.3
<i>proteus spp</i>	4.5	ND	ND	1.5
<i>Citrobacter freundii</i>	12.7	15	28.3	18.7
<i>Acintobacter SPP</i>	16.6	19	16.7	17.4
<i>Aeromonas SPP</i>	0	19.6	10	9.9

Isolation and identification of bacteria

The cultured samples resulted in the identification of three major groups of bacteria: Gram Positive Cocci 91 %, Gram Positive Bacilli 2 % and Gram Negative Bacilli 5.5 % and unidentified bacteria 1.5% (Figure 1). Gram Negative Bacilli comprised 2.35%,

1.57% and 1.58% in OT, ICU and NICU, respectively (Table 2). These results may related to the ability of Gram Positive Bacteria to survive much longer in the aerosolized state and in a dry environment than Gram Negative Bacteria., which can only survive a short time without moisture.^{9,11,22}

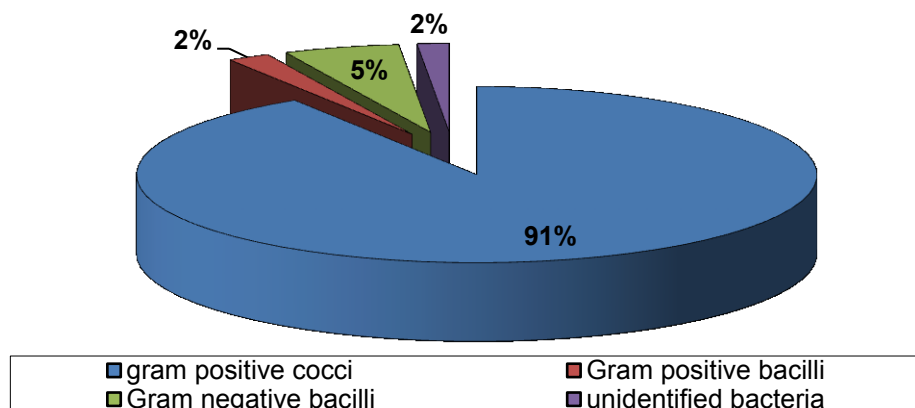


Figure 1: Distribution of isolated bacteria.

From all Gram Negative Bacilli *Pseudomonas aeruginosa*, *Citrobacter freundii* and *Acintobacter spp.* comprised of 42.8%, 18.7% and 17.4% respectively in all studied units (Figure 2). However, *Aeromonas spp.*

comprised of 10% which present only in ICU and NICU. Each of *Enterobacter cloacae*, *E. sakazakii* and *Proteus spp.* comprised 3.4%, 6.3% and 1.5% respectively they present in OT only.

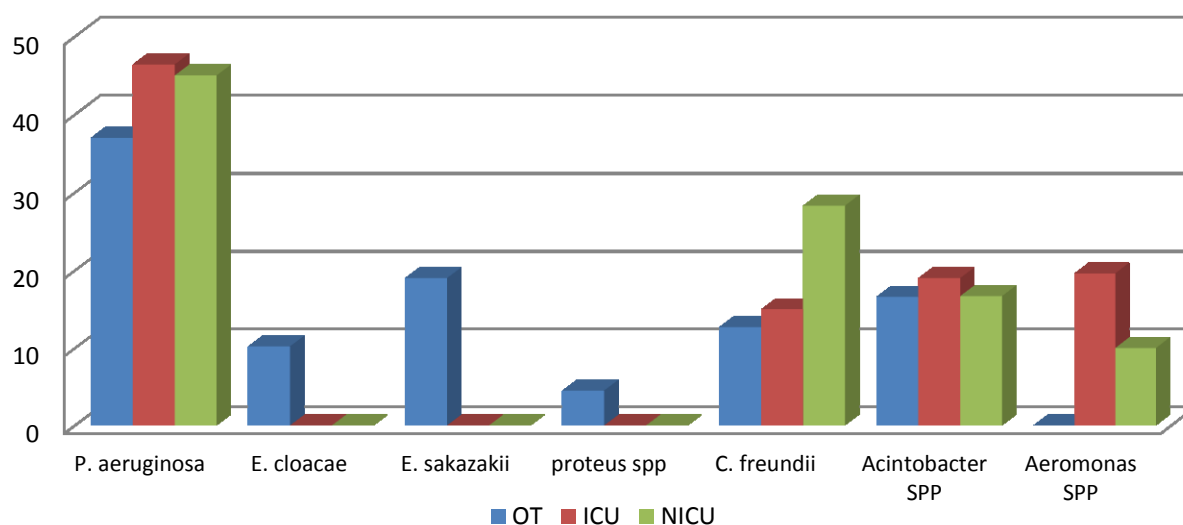


Figure 2: Distribution and Percentage of for Gram Negative Bacilli in

P. aeruginosa were the major Gram negative bacilli isolated from the air of all studied units of all hospitals. This could be due to its associated with different type of human infections. Moreover, they can be difficult to eradicate from hospital wards, as it is resistant to, and multiply in, many of the disinfectants

and antiseptics commonly used in hospitals.²² Chandrashekar et al¹⁸ and Panagea et al²¹ reported that *P. aeruginosa* were the major Gram negative bacilli in the NICU, and the presence of *Citrobacter spp.* similar to our study.

Other types of opportunistic Gram Negative Bacilli such as *Acinetobacter* spp., *Enterobacter* spp., *Citrobacter* spp., *Aeromonas* spp. were isolated from air in all studied units as has been reported before at higher or lower percentages.^{16,18,22,29} *Enterobacter sakazakii* is being reported in this study for the first time about hospitals air. This could be due to the dust contamination. The presence of sex bacterial types of seven in OT is a good indicator to large number and type of cases and to bad sterilization systems in OT.

The ability of *P. aeruginosa*, *Acinetobacter* spp., *Citrobacter* spp. and *Aeromonas* spp. to colonization in hospitals environment, survive under harsh condition and developed antimicrobial resistance^{16,22,26} are the possible reasons for its high percentage in this study. Also antibiotics use for long duration of hospitalization, cases of patents and ICU indwelling devices and equipments could be a cause for increase in percentage of isolated pathogens.^{19,23,30}

Antimicrobial susceptibility profiles

The different antibiotics that are commonly used in Jordan were evaluated against all of the

isolated Gram Negative Bacilli bacteria (Table 3). Data revealed that the higher percentage resistance of Gram Negative Bacilli was 25.1% to Ceferioxone, 24.6% to Nalidixic acid, 22.6% to Cefuroxime and Cefaclor. Moreover, the lowest resistance rate was observed with Gentamycin 9.4%, Ciprofloxacin 10%, Imipenem 10.4%, amikacin 11.4% (Figure 3), these results constant with NCCLS recommendations 2003 for Gram Negative Bacilli treatment (27). These four antibiotics that have a lowest resistance may play an important role to prevent nosocomial infection in Jordan hospitals. The higher percentage resistance of Gram Negative Bacilli may due to the extensive use of these antibiotics for long time.

This study suggests that the monitoring strategies should be designed towards the continual monitoring of the presence and distribution of these organisms in the hospitals environment, furthermore cleaning products and adequate ventilation (and filtration) systems should be controlled and monitored consensually.

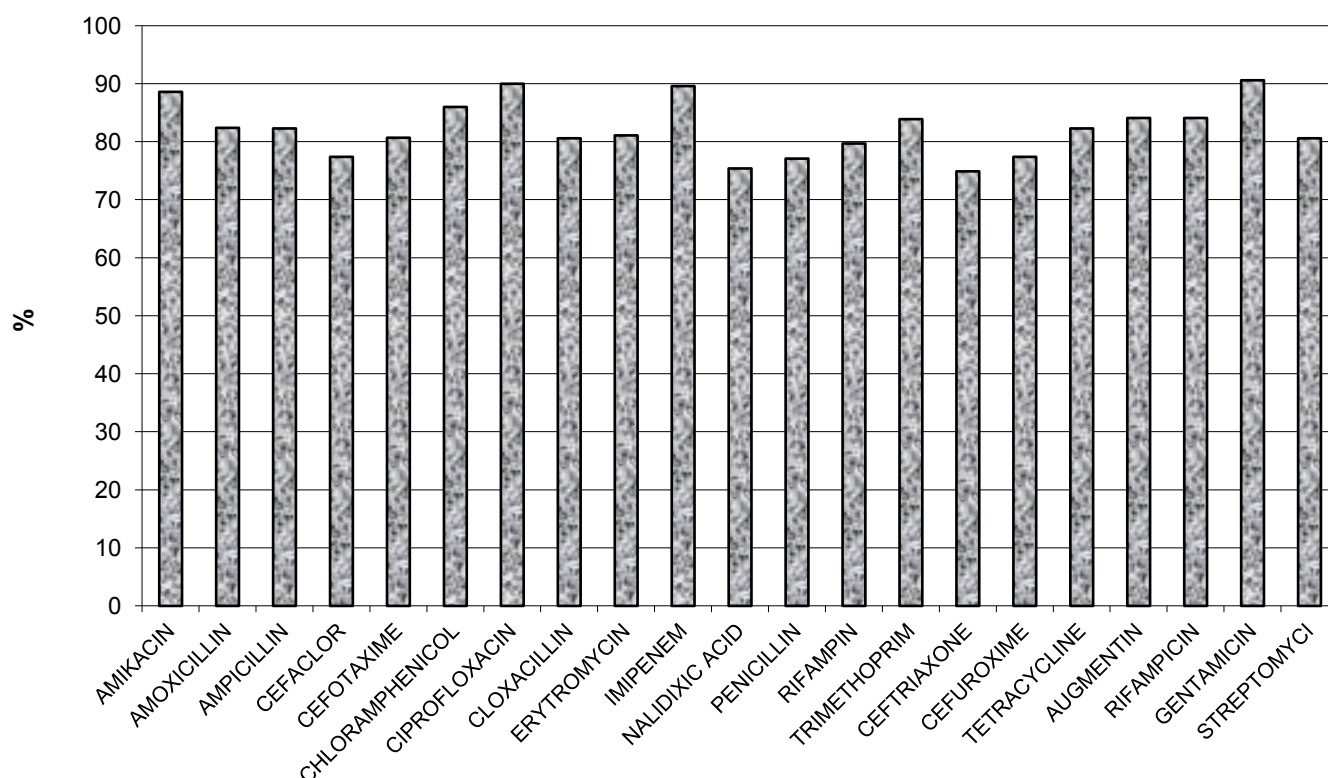


Figure 3: Antimicrobial Susceptibility Pattern of Gram Negative Bacilli Isolated during the study.

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