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EPIDEMIOLOGIC CHARACTERISTICS OF *KLEBSIELLA PNEUMONIAE* ISOLATES IN VENTILATOR-ASSOCIATED PNEUMONIA IN INTENSIVE CARE UNITS

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ABSTRACT
***Klebsiella pneumoniae* is a common pathogen that causes ventilator associated pneumonia (VAP) in intensive care units (ICUs). Strain typing is a useful tool in tracking the spread of these infections. Primary objective was to study different strains causing VAP in Anesthesia ICUs. Secondary objective was to determine role of health-care workers (HCWs) and ICU environment in the transmission of these strains. Endotracheal aspirates of 60 VAP patients, surveillance samples from the HCWs (18)and the ICU environment (193)were collected. Antibiogram typing and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) were used for comparison of the isolates from VAP patients and surveillance samples. Antibiogram showed 5 antibiotic susceptibility patterns that were designated A1-A5. ERIC-PCR yielded 1 to 5 amplification bands. All the isolates were typable by ERIC-PCR. Eight ERIC patterns were obtained ERIC(I)-ERIC(VIII). ERIC-PCR typing method gave higher discriminatory index (D) (0.7557) than antibiogram (0.6035). There was sharing of certain ERIC patterns among patient and HCWs or environmental sources. In Conclusion: *K.pneumoniae* is the most dominant pathogen in anesthesia ICUs. Throats and hands of HCWs are possible sources of pathogen transmission to patients. Surfaces with hand contact of the medical staff are often contaminated and may serve as vectors for cross transmission.**

Key words: Ventilator-associated pneumonia, ICU environment, health-care workers, *Klebsiella pneumoniae*, antibiogram typing, ERIC-PCR

LES CHARACTERISTIQUES EPIDEMIOLOGIQUES DES ISOLATS DE *KLEBSIELLA PNEUMONIAE* DANS LA PNEUMONIE ASSOCIEE AU VENTILATEUR EN UNITES DE SOINS INTENSIFS.

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RESUME

***Klebsiella pneumoniae* est un pathogène commun qui cause la pneumonie associée au ventilateur (VAP) dans les unités de soins intensifs (ICUs). Le typage de souche est un outil utile pour suivre la propagation de ces infections. L'objectif principal était d'étudier les différentes souches qui causent le VAP en anesthésieICUs. L'objectif secondaire était de déterminer le rôle des professionnels de la santé (HCWs) l'environnement des soins intensifs dans la transmission de ces souches. Aspiration endotrachéale de 60 patients de VAP, des échantillons de surveillance des travailleurs de la santé de l'environnement des soins intensifs ont été recueillis. Le typage antibiogramme et le consensus inter génique répétitifentérobactérieréaction en chaîne par polymérase (ERIC – PCR) ont été utilisés pour la comparaison des isolats des patients VAP et des échantillons de surveillance. L'antibiogramme a montré 5 modèles de susceptibilité aux antibiotiques qui ont été désignés A1 – A5. ERIC – PCR a donné 1 à 5 bandes d'amplification. Tous les isolats ont été typable par cette méthode. Huitmodèles ERIC ont été obtenusERIC(I)-ERIC(VIII). Le typage méthode d'ERIC – PCR a donné un indice discriminatoire plus élevé (D) (0,7557) que l'antibiogramme (0,6035). Il y avait le partage de certains schémas ERIC chez les patients et les travailleurs de la santé ou des sources environnementales. En conclusion, *K.pneumoniae* est le pathogène le plus dominant en anesthésie des unités de soins intensifs. Les gorges et les mains des travailleurs de la santé sont des sources possibles de transmission de pathogènes aux patients. Les surfaces à contact manuel du personnel médical sont souvent contaminées et peuvent servir de vecteurs pour la transmission transversale.**

Mots clés : Pneumonie associée au ventilateur, l'environnement de soins intensifs, les travailleurs de la santé, *Klebsiella pneumoniae*, typage antibiogramme, ERIC – PCR.

INTRODUCTION

Ventilator-associated Pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after the initiation of endotracheal intubation and mechanical ventilation (MV) **(1).** Several studies have shown that critically ill patients are at high risk for getting such infection and so it continues to be a major cause of morbidity, mortality and increased financial burden in ICUs **(2)**. Health-care workers (HCWs), contaminated equipment, and the ICU environment have been implicated in health-care associated outbreaks. *K. pneumoniae* is very well adapted to the hospital environment since it exhibits higher survivability on hands and environmental surfaces than other *Enterobacteriaceae* **(3)**. Cross-transmission can also occur from patient to patient via hands of the HCWs **(4).** Strain typing by traditional phenotypic methods may lack discriminatory power and stability. Molecular techniques offer a considerable improvement, and can complement phenotypic data to obtain a better understanding of bacterial diversity **(5)**.

Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) is a simple, high throughput, affordable, reproducible, and discriminatory molecular typing method. Furthermore, it has excellent sub typing results and does not require much skill to perform **(6).**
Because of the tremendous diversity of bacterial genomic DNA, sequences of ERIC-PCR bands are often unique to the genome of the strain used for amplification. Therefore, these sequences have been used to design primers for discriminating closely related bacterial strains **(7).**

METHODS

Study design: This prospective study was conducted from December 2012 to February 2014 in Medical Microbiology and Immunology Department and Anesthesia Intensive Care Units (ICUs), Zagazig University Hospitals. There are 15 beds separated by curtains in each of the two anesthesia ICUs with adequate space for movement of staff and equipment.
Ethical consideration: Approval for performing the study was obtained from the Institutional Review Board (IRB) and Medical Microbiology and Immunology Department and Anesthesiology and Intensive Care Department, faculty of medicine, Zagazig University.
Study population: This study included 60 patients who were suspected clinically to have ventilator associated pneumonia **(8)**. Demographic and procedure-related information were collected.
Collection of samples

A) Patients' samples: According to the method described by **Karen and co-workers (9)**; EA samples were collected from the patients early in the morning in screw-capped, sterile, wide mouthed plastic containers.

B) Health-care workers' samples:

Throat samples: They were collected from health-care workers (HCWs) who were requested not to take any antibiotic or mouth-washes eight hours before swabbing **(10).**

Hand impressions: They were requested to press their fingers onto blood agar plates. Sampling was performed at midday, by which time staff members had been in contact with patients for several hours **(3).**

C) Environmental samples: According to the results of patients samples, environmental samples were taken throughout the ICUs, concentrating on surfaces and areas with maximum potential for hand contact and cross-infection. A total of 175 samples; 25 were taken from the following; ventilator tube, ventilator screen, humidifier fluid, suction apparatus, bed rail, over bed and medicine trolley. The lumen of the ventilator tube and the humidifier fluid container were swabbed by rubbing sterile cotton swab sticks, against the inner wall of both of them in a horizontal, then vertical, and then diagonal direction several times then the swabs were rolled to expose unused sides. On the other hand, surfaces of the ventilator screen, suction apparatus, bed rail, over bed and medicine trolley were also swabbed with sterile cotton swab sticks, pre-moistened with peptone water **(11).**
Regarding air sampling, samples were collected from air in the ICUs starting from June 2013 during collecting the patients' samples, by agar settle plates method, where blood agar plates were left open to the air according to the 1/1/1 scheme (for one hour, at a height of one meter at least one meter from walls) **(12)** and compared to other plates left open for 24 hours **(13).**

Transport of samples: All samples were transported to the laboratory within one hour of sampling process. The environmental swabs were inoculated within one hour in enriched brain heart infusion (BHI) broth and incubated for 24 hours at 37°C **(9)**.

Samples processing: Endotracheal aspirates were examined microscopically by Gram's stain and 10µL were streaked on MacConkey

medium in four-quadrants consecutively, then incubated at 37°C for 24 hours. Interpretation was as the following; growth was classified as rare (1+), light (2+), moderate (3+), or heavy (4+), based on the number of colonies in each quadrant, (3+) grade was considered diagnostic for VAP **(14).** Microbiological confirmation of suspected VAP cases was based on a positive Gram stain (≥25 pus cells/low power field and ≥1 bacteria/oil immersion field) **(15)** and semi-quantitative endotracheal aspirate (EA) cultures of moderate (3+) or heavy growth (4+), where (3+) is equivalent to quantitative culture showing $\geq 10^5$ colony forming unit (CFU)/ml **(1).**

Throat swabs of health-care workers' were streaked out on blood agar and MacConkey agar plates. Then, they were incubated aerobically at 37°C for 24 hours **(16).** Blood agar plates of hands impression were also incubated at 37°C for 24 hours **(3).**

Environmental swabs were streaked out on blood agar and MacConkey agar plates. Then, they were incubated aerobically at 37°C for 24 hours **(16).** Blood agar plates of air samples were also incubated aerobically at 37°C for 24 hours **(9).**

Identification of *K. pneumonia* isolates: The isolates were identified according to the results of colonial morphology, microscopic examination of Gram-stained films, and conventional biochemical reactions including: oxidase test, action on triple sugar iron medium, indole test, methyl red test, Voges-Proskauer test, citrate utilization test, urease test and motility test **(17).** API 20 E strips **(Bio-Mérieux, USA)** were used for confirmation of some suspected isolates which were positive for indole-production.

Maintenance of the selected isolates:
The selected isolates that fulfilled the criteria of being *K. pneumoniae* were inoculated on nutrient agar slopes. After an overnight incubation at 37°C, the slopes were kept at 4°C. Subculturing of the isolates was done every 2-3 weeks. Also, before starting any experiment, subculture was done twice to allow the cells to restore its viability.

The study was conducted on 60 patients admitted to the ICUs and diagnosed as having VAP. They were 34 males and 26 females and their ages ranged from 18 to 75 years old (X±SD: 49.05±14.8). Out of the 60 patients' endotracheal aspirates, 9 (15%) showed no growth on MacConkey agar plates while 51 (85%) were Gram negative, of them; 9 (17.6%) were only colonized as they showed rare (1+)

Antibiotic susceptibility testing: Antibiogram typing was performed by the Kirby–Bauer disc diffusion method **(18).**The diameters were interpreted as Resistant, Intermediate, and Susceptible according to CLSI published diameters **(19).**

ERIC-PCR typing: For comparison of the isolates from the surveillance samples and VAP patients, ERIC-PCR was used. DNA extraction was done using G-spin™ Total DNA Extraction Mini Kit **(iNtRON Biotechnology, Korea)**. The supernatant containing DNA in the tubes were stored at - 20°C until being used.

ERIC-PCR was performed using PCR Premix **(iNtRON Biotechnology, Korea)**. Two primers were used (**Biolegio, Netherlands)**; ERIC1 and ERIC2 were designed according to **Versalovic and co-workers (20)** as; ERIC1: 5' ATG TAA GCT CCT GGG GAT TCA C 3'; ERIC2: 5′ AAG TAA GTG ACT GGG GTG AGC G 3′. ERIC-PCR was performed in a final volume of 20 µL containing 2 µL of the template DNA, 1 µL of primer ERIC1R (10pmol/µL), 1 µL of primer ERIC2 (10pmol/µL), 16 µL distilled Water. Each reaction mixture was amplified with a heated lid thermal cycler **(Biometra, UK)**.
Reaction conditions were as follows: 94°C for 1 minute, followed by 35 cycles at 94°C for 30 seconds, 25°C for 30 seconds, 72°C for 1.5 minutes, and a final extension at 72°C for 10 minutes **(21).** The amplified PCR products in parallel with a DNA molecular size marker that gave 11bands ranging from 100-1500 base pairs **(iNtRON Biotechnology, Korea)** were detected by agarose gel electrophoresis as described by **Viljoen and co-workers (22).** The gel was carefully removed and was viewed and photographed over the UV transilluminator **(Biometra, UK)**.

STATISTICAL ANALYSIS

The data were coded, entered and checked using the Statistical Package for Social Science (SPSS) software system (Version 11.0; Chicago, IL). The numerical discriminatory index (D) which is a measure of the discriminatory ability of the typing methods was calculated according to Hunter **(23)**.

RESULTS
and light (2+) growth by semi-quantitative culture, while 42 (82.4%) patients were infected and showed moderate (3+) and heavy (4+) growth. The infection was polymicrobial in 22 (52.3%) patients and monomicrobial in 20 (47.7%). Total number of isolated Gram negative organisms was 64 isolates.
The study showed that the frequency of *K. pneumoniae* isolation among Gram negative

bacilli isolates from VAP patients was 25/64 (39%) and that of HCWs throat and hand samples was 3/18 (16.7%) and 2/18 (11.1%); respectively. Regarding environmental and air samples, frequency of isolation was 44/ 175(25.2%) and 2/18 (25%); respectively. Highest frequencies of *K. pneumoniae* isolation from environmental samples were from ventilator tube 11/25(44%), humidifier fluid 11/25(44%) and ventilator screen 8/25(32%).
Results of antibiotic susceptibility testing of *K. pneumoniae* isolates were shown in (**Table 1).**

There were 5 antibiotic susceptibility patterns that were designated A1-A5. All the five patterns showed multidrug resistance (MDR) as strains were resistant to 5 or 6 antibiotics. The most alarming patterns were A4 and A5 as strains belonging to A4 were only sensitive to amoxicillin/clavulinic acid, imipenem and colistin. Also, A5 was the only pattern that showed resistance to amoxicillin/clavulinic acid, imipenem and colistin among all other patterns, in addition to its resistance to tobramycin, cefotaxime, ceftazidime and ampicillin **(Table 2).**

TABLE (1): RESULTS OF ANTIBIOTIC SUSCEPTIBILITY TESTING

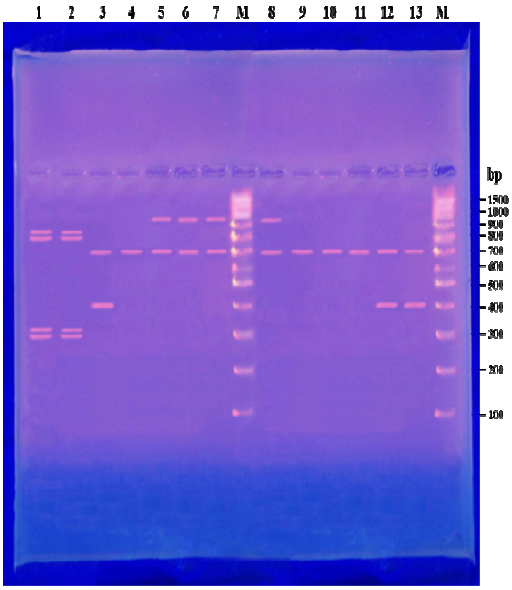
	Resistant *n* (%)	**Sensitive *n* (%)**
Amikacin	**22 (28.9)**	**54 (71.1)**
Gentamycin	**50 (65.8)**	**26 (34.2)**
Ampicillin	**76 (100)**	**0**
Piperacillin	**68 (89.5)**	**8 (10.5)**
Ceftazidime	**41 (53.9)**	**35 (46.1)**
Ceftriaxone	**76 (100)**	**0**
Amoxicillin/clavulinic acid	**8 (10.6)**	**68 (89.4)**
Cefotaxime	**76 (100)**	**0**
Ciprofloxacin	**39 (51.3)**	**37 (48.7)**
Tobramycin	**76 (100)**	**0**
Colistin	**8 (10.6)**	**68 (89.4)**
Imepinem	**8 (10.6)**	**68 (89.4)**
Total	**76 (100)**	

Figure 1 shows similarity between lane 1, for an over bed isolate and lane 2, for a ventilator tube isolate. Lanes 3, 12 and 13 for a patient isolate, an isolate from his ventilator tube, and an isolate from his humidifier fluid respectively show similarity. There is similarity among lanes 4, 9, 10 and 11 for a patient isolate, a ventilator screen isolate, hand of health-care worker isolate, and throat of health-care worker isolate respectively. Also, lanes 5, 6, 7 and 8 are for a patient isolate, an isolate from his ventilator tube, an isolate from his humidifier fluid and an isolate from his suction apparatus show similarity.
Figure 2 shows the eight ERIC-PCR patterns that were observed from the results. These patterns were designated ERIC(I)-ERIC(VIII). They yielded 1 to 5 amplification bands, where the size of amplified DNA bands ranged from 100 bp to 1000 bp.

TABLE (2): OBSERVED PATTERNS OF ANTIBIOTIC SUSCEPTIBILITY FOR ISOLATED *K. PNEUMONIAE* STRAINS.

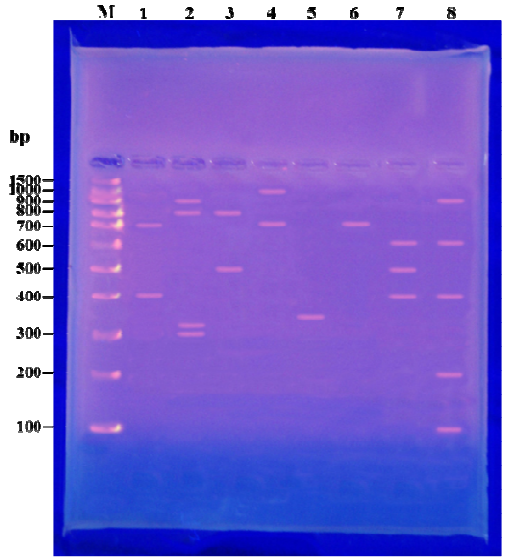
	A1	A2	A3	A4	A5
AK	S	S	S	**R**	S
CN	**R**	S	S	**R**	S
CIP	**R**	S	S	**R**	S
PRL	**R**	**R**	**R**	**R**	S
TOB	**R**	**R**	**R**	**R**	**R**
CTX	**R**	**R**	**R**	**R**	**R**
CAZ	S	**S**	**R**	**R**	**R**
AMP	**R**	**R**	**R**	**R**	**R**
IPM	**S**	S	**S**	**S**	**R**
CT	**S**	**S**	**S**	**S**	**R**
AMC	**S**	**S**	**S**	**S**	**R**
CRO	**R**	**R**	**R**	**R**	**R**
***n* (%)**	**18 (23.7)**	**18 (23.7)**	**10 (13.2)**	**23 (30.3)**	**7 (9.1)**

FIGURE 1: ETHIDIUM BROMIDE-STAINED AGAROSE GEL SHOWING RESULTS OF ERIC-PCR FOR ISOLATED *K.*



***PNEUMONIAE* STRAINS Lanes M: Molecular size marker which gave 11bands ranging from 100-1500bp. Lanes 1, 2: The size of amplified DNA bands is 300, 320, 800 and 900bp. Lanes 3, 12, 13: The size of amplified DNA bands is 400 and 700bp. Lanes 4, 9, 10, 11: The size of amplified DNA bands is 700bp. Lane 5, 6, 7, 8: The size of amplified DNA bands is 700 and 1000 bp.**

FIGURE 2: DIFFERENT OBSERVED ERIC-PCR PATTERNS FOR ISOLATED *K.PNEUMONIAE*



STRAINS Lanes M: Molecular size marker which gave 11bands ranging from 100-1500 bp. ; Lane 1: The size of amplified DNA bands is 400 and 700 bp. Lane 2: The size of amplified DNA bands is 300, 320, 800 and 900 bp. ; Lane 3:The size of amplified DNA bands is 500 and 800 bp. Lane 4: The size of amplified DNA bands is 700 and 1000 bp.; Lane 5: The size of amplified DNA bands is 340 bp. Lane 6:The size of amplified DNA bands is 700 bp.; Lane 7: The size of amplified DNA bands is 400, 500 and 600 bp. Lane 8: The size of amplified DNA bands is 100, 200, 400, 600 and 900 bp

ERIC-PCR typing method gave higher discriminatory index (D) (0.7557) than antibiogram (0.6035) (**Table 3**). By analyzing ERIC-PCR typing data, possible epidemiological linkages were proven **(Table 4)**.

TABLE (3): COMPARISON BETWEEN ANTIBIOGRAM AND ERIC-PCR

	No. of different patterns	No. of strains belonging to the most numerous pattern	Numerical discriminatory index
Antibiogram	5	23	0.6035
ERIC-PCR	8	18	0.7557

TABLE (4): EPIDEMIOLOGICAL ANALYSIS OF TYPING DATA

	Source	Antibiotic pattern	ERIC pattern
p1, p4, p5, p16	Patient	1	I
e1, e5, e23, e28	Ventilator tube	1	I
e2, e6, e7	Humidifier fluid	1	I
e22, e30	Ventilator screen	1	I
t3	Throat	1	I
a1	Air	1	I
e12	Ventilator tube	1	II
e18	Humidifier fluid	1	II
e42	Over bed	1	II
p2, p3, p8, p10, p11, p17, p18, p21	Patient	2	III
e3, e32	Ventilator tube	2	III
e4, e14	Bed rail	2	III
e13, e16	Ventilator screen	2	III
e15	Over bed	2	III
e17	Suction apparatus	2	III
e31	Humidifier fluid	2	III
e38	Medicine trolley	2	III
p6, p12, p15	Patient	3	IV
e20, e26	Ventilator tube	3	IV
e10, e21, e27	Humidifier fluid	3	IV
e8	Ventilator screen	3	IV
e9	Suction apparatus	3	IV
p7, p13, p22, p23	Patient	4	V
e11, e40	Bed rail	4	V
e24	Medicine trolley	4	V

	Source	Antibiotic pattern	ERIC pattern
e29, e35, e37	Humidifier fluid	4	V
e39	Suction apparatus	4	V
e41	Ventilator screen	4	V
p24, p25	Patient	4	VI
e43	Ventilator screen	4	VI
e44	Bed rail	4	VI
h1, h2	Hand	4	VI
a2	Air	4	VI
t1, t2	Throat	4	VI
e19	Over bed	4	VII
p9, p14, p19, p20	Patient	5	VIII
e25	Suction apparatus	5	VIII
e33	Ventilator screen	5	VIII
e34	Ventilator tube	5	VIII

KEY: p: patient endotracheal aspirate, e: environmental swab, t: throat swab of health-care worker, h: hand impression of health-care worker, a: air sample.

DISCUSSION

In spite of significant changes in the spectrum of organisms causing VAP, *K. pneumoniae* has held a nearly unchanged position as an important pathogen **(24).**

In the present study, we reported that the frequency of isolation of *K. pneumoniae* was the highest one; 25/64(39%). This is in accordance with that of a World Health Organization (WHO) cooperative study involving 55 hospitals in 14 countries where there was a predominance of Gram-negative pathogens causing VAP, *K. pneumoniae* was diagnosed in 40% of cases **(25).**

In addition, a relatively closer result was that of Set and co-workers **(26)** who isolated it from 33.3% of VAP patients from a tertiary care center in Mumbai. Also, in a Cairo University hospitals surveillance program by El-Kholy and co-workers **(27)** where it was 29.2% and by Krishnamurthy and co-workers **(15)** whose frequency was 24.78%.

Research into the frequency of contact of ICU patients with the medical staffs revealed that the medical staffs were in direct contact with patients 159 times per day and experienced indirect contact with patients 191 times per day **(28).**

In this study, we expected that one of the possible causes of transmission of infection with *K. pneumoniae* to the ICU patients was HCWs, as the organism was isolated from 3/18(16.7%) of their throat samples and 2/18(11.1%) of their hand samples. This might be due to inadequate application of standard precautions for infection control and hand hygiene measures.

Gupta co-workers **(29)** also found a dominant strain of *K. pneumoniae* on the hands of two medical staff in their investigations into the outbreak of *K. pneumoniae* in a neonatal intensive care unit (NICU).

In the present study the environmental sampling had shown that 44/175(25%) of the samples were positive for *K. pneumoniae* which is slightly higher than the result of Daef and co-workers **(30)** which was 16.4%. This figure reflected the fact that *K. pneumoniae* is ubiquitous in the hospital environment. These sites were ventilator tube 11/25(44%), humidifier fluid 11/25(44%), ventilator screen 8/25(32%), bed rail 5/25(20%), suction apparatus 4/25(16%), over bed 3/25(12%) and medicine trolley 2/25(8%).

In accordance with our results, Narciso and associates **(31)** isolated 2 strains from ventilator screen and suction device. *K. pneumoniae* was also isolated from 3.5% of suction apparatus samples and 5.6% of medicine trolley samples in NICU **(32)**.

Das and co-workers **(33)** pointed out that the presence of *K. pneumoniae* in air might be attributed to the bacterial aerosols generated due to coughing and sneezing. In the present study, no growth of *K. pneumoniae* obtained from agar plates after leaving them open for 1 hour, unlike obtaining 2 out of 8 *K. pneumoniae*

growth after leaving them open for 24 hours. This finding matched with that detected by Krishna and colleagues **(34)** who found that all air samples collected from NICU of Karnataka institute of Medical Sciences hospital in India were negative for *K. pneumoniae*, where the air sampling was done using settle plates exposed to the NICU air for only ½ an hour.

Calculating the numerical discriminatory (D) index for ERIC and antibiogram demonstrated that ERIC typing (0.7557) was more discriminatory than antibiogram (0.6035). This is in agreement with Freitas and Barth **(35)** who declared that the low discriminatory power of susceptibility tests was not surprising since the power of a method was determined by the number of types defined by it and the relative frequencies of these types.

In a study done by Mansour and colleagues **(36)**, ERIC typing gave a higher D index than antibiogram in Egypt and Saudi Arabia (0.801 and 0.785 respectively). By analyzing various typing data, we detected some possible epidemiological linkages; sharing of certain ERIC patterns among patient strains that may be explained by horizontal transmission from patient to another patient, probably from the hands of HCWs or environmental sources.

A direct link among two hand strains, two throat strains and two patients' strains, belonging to ERIC(VI) genotype was proven. In addition, a direct link among one throat strain and four patients' strains, belonging to ERIC(I) genotype was proven.

Ventilator tubes, humidifier fluid and ventilator screen had a central role in the spread of *K. pneumoniae* in the ICU. Epidemiological linkage was proven among patients and ventilator tubes by harboring strains belonging to ERIC(I), ERIC(III), ERIC(IV), ERIC(V) and ERIC(VIII) genotypes.

Regarding linkage among patients and humidifier fluid, both of them harbored strains belonging to ERIC(I), ERIC(III), ERIC(IV) and ERIC(V) genotypes. It may be explained by that fluid reservoir of the humidifier fluid may have been filled by non-sterilized water.

Epidemiological linkage was also proven among patients and suction apparatus by harboring strains belonging to ERIC(III), ERIC(IV), ERIC(V) and ERIC(VIII) genotypes. This might be explained by failure of sterilization of suction apparatus tubing and inadequate application of standard precautions for infection control.

Evacuation of suction apparatus fluid into drainage containers is a possible reason that could explain its linkage to bed rails and medicine trolley by harboring strains belonging to ERIC(III) and ERIC(V) genotypes, where any surface could has been contaminated by fluid spillage. Sharing of ERIC(I) and ERIC(VI) among ventilator screen, air, throats and hands of HCWs could be possibly explained by aerosols generated due to coughing or sneezing and hand contact where *K.pneumoniae* can survive on inanimate surfaces even for months.

The utilization of typing methods to draw possible epidemiological transmission linkage was done previously in other studies, for example, an outbreak caused by a multidrug-resistant *K. pneumoniae* (MRKP) strain occurred in a Tunisian neonatal ward, ERIC-PCR combined with other typing methods showed spread of at least two epidemic strains within the ward **(37)**.

An outbreak caused by a MRKP strain occurred also in the ICU of the St. Elisabeth Hospital in Tilburg, The Netherlands, using molecular typing, confirmed similarity of the isolates to those recovered from the roll boards **(38)**.

From the results obtained from this study, we recommend strict adherence to environmental infection control measures that is essential to prevent health-care-associated infections. Also, we recommend use of disposable suction tubes or properly disinfecting them every single suction for a patient. In addition, compliance of health-care workers to hand hygiene measures should be monitored. Use of face mask by health-care workers when manipulating patients or respiratory equipment to prevent droplet and bacterial particles transmission to patients must be applied. On the other hand, using (ERIC-PCR) typing method, which is proven to be superior to antibiotic typing in tracing source of infection, is recommended.

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