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PREVALENCE OF *KLEBSIELLA PNEUMONIAE* CAUSING VENTILATOR-ASSOCIATED PNEUMONIA (VAP) ISOLATED FROM PEDIATRIC INTENSIVE CARE UNIT AT ASSIUT UNIVERSITY HOSPITAL

Eman Ramadan Mohamed[*1], Hamada Mohamed Mohamed[1], Sherine Ahmed Abd El- Rahman[2] and Shabaan Ahmed Hashem[2]

[1]*Department of Microbiology and Immunology, Faculty of pharmacy, Al –Azhar University, Assiut branch*
[2]*Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt*

Ventilator-associated pneumonia is a common nosocomial infection occurring in patients receiving mechanical ventilation especially in pediatric intensive care units (PICUs). Klebsiella pneumoniae is a multidrug resistant nosocomial pathogen that plays an important role in respiratory tract infection among critically ill patients.

This study included 51 pediatric patients had VAP admitted to the Pediatric Intensive Care Unit (PICU) at Assiut university children's hospital through 12-month period from May 2014 to May 2015, using quantitative endotracheal aspirate (EA) culture. K. pneumoniae were isolated by culturing onto blood and MacConkey`s agar plates then incubated aerobically at 35°C for 24-48 hrs. and identified by morphology and biochemical tests. K. pneumoniae 19 (37.25%) was the most isolated bacteria that causing VAP in PICU. Antimicrobial susceptibility testing showed that K. pneumoniae isolates were resistant to all commercial available antimicrobial agents that used in the PICU.

INTRODUCTION

Ventilator-associated pneumonia is one of the most common hospital-acquired pneumonias occurring in patients receiving mechanical ventilation that develops 48 hours or more after the initiation of ventilation[1]. It is the second most common nosocomial infection in pediatric intensive care units (PICUs). Such infection adversely affects patient outcome and results in considerable morbidity and mortality. It also significantly increases medical costs by prolonging PICU and hospital stay[2].

Klebsiella pneumoniae is a common bacterial pathogen involved in hospital-acquired pneumonia as well as diverse healthcare-associated infections, such as blood stream infection, urinary tract, post-surgical, and intensive care-related infections[3]. *Klebsiella pneumoniae* is one of the most important bacteria which cause nosocomial infections, especially at pediatric units[4].

K. penumoniae is Gram-negative, non-motile and usually produce a prominent acidic polysaccharide based capsules. Biochemical characteristics used for the identification of *K. pneumoniae* include: negative indole-test, production of lysine decarboxylase (but not ornithine decarboxylase), fermentation of specific sugars (e.g. D-glucose, lactose, sucrose, Larabinose and maltose) and sugar-alcohols (e.g. D-mannitol)[5].

The aim of our study is to estimate the prevalence of nosocomial VAP caused by *Klebsiella pneumoniae* in PICU at Assiut University Children's Hospital and evaluate antimicrobial resistance patterns of the isolates.

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*Corresponding author: Eman Ramadan Mohamed, E-mail: eman-ramadan86@aun.edu.eg

MATERIAL AND METHODS

Study population

This study was conducted over a period of 12 months from May 2014 to May 2015. Fifty-one (51) endotracheal aspirate specimens were obtained from ventilator-associated pneumonia (VAP) infected patients admitted to the Pediatric Intensive Care Unit (PICU) at Assiut university children's hospital.

Bacteriological Examination

All samples were inoculated on blood agar and macConkey's agar and incubated at 37°C for 24-48 hrs. Significant bacterial count was considered $\geq 10^6$ CFU/ml, suspected colonies were sub-cultured on Eosin Methylene Blue (EMB) agar.

Identification and confirmation of isolates was done by Gram stain, colony morphology, oxidase test, triple sugar iron test (TSI), simmon's citrate, christensen's urea, voges-proskauer and motility, indole, ornithine (MIO) test.

Further confirmation and biotyping of *K. pneumoniae* isolates by API20E kit

Analytical Profile Index (API) is a biochemical panel for identification and differentiation of members of the family *Enterobacteriaceae*.

Antimicrobial susceptibility patterns of *K. pneumoniae* isolates

The antimicrobial susceptibility of the isolated *K. pneumoniae* was done by the Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines[6].

The following antimicrobial discs were used (Hi-Media laboratories, BD Diagnostics Pvt Ltd, India): Piperacillin (100 µg), Amoxacillin-Clavulanic acid (20, 10 µg), Amikacin (30 µg), Gentamicin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Cefazolin (30 µg), Cefoperazone (75 µg), Ceftriaxone (30 µg), Cefipeme (30 µg), Ceftazdime (30 µg), Levofloxacin (5 µg), Colistin (10 µg), Trimethoprim /Sulphamethoxazole (1.25/23.75 µg).

RESULTS AND DISCUSSION

Results

Fifty-one endotracheal aspirates specimens were collected from ventilator-associated pneumonia (VAP) infected patients admitted to the Pediatric Intensive Care Unit (PICU) at Assiut university children's hospital. Out of 51 VAP infected patients, 32 (62.7%) were males and 19 (37.3%) were females. The age of patients with nosocomial VAP infection ranged from one month to 14 years old. Seventy percent (35) of these cases were infant patients aged 1-12 months, 11.8% (6) among toddler patients aged >1to<3 years, 9.8% (5) among pre-school children aged >3 to <5 years, 5.9% (3) among school children aged 5-10 years while 2% (1) of cases were among patients aged 11-14 years, as presented in table 1.

Table 1: Age distribution of patients with nosocomial VAP infection.

Age of patients	No. n=51	%
Infant: 1-12 months	36	70
Toddler: 1-3 years	6	11.8
Pre-school: 3-5 years	5	9.8
School-age: 5-10 years	3	5.9
Adolescent:11-18 years	1	2

Clinical indications of mechanical ventilation in the studied patients

The clinical indications of mechanical ventilation in the patients with nosocomial VAP infection (Table 2). These included metabolic causes in 5 patients (9.8%), as renal failure and malignant hypertension. Pulmonary diseases as severe bronchial asthma, acute respiratory distress and chronic obstructive pulmonary disease were the cause in 13 patients (25.49%). Cardiac failure was in 2 patients (3.92%). Neurologic compromise as Gillian bare syndrome, intracranial hemorrhage and convulsion were the indications in 24 patients (47.05 %). Genetic disease as Down syndrome were in 3 patients (5.88%). Post–surgical patients were in 4 patients (7.84%).

Table 2: Indications of mechanical ventilation in the patients with nosocomial VAP infection.

Indications of Mechanical Ventilation	No. n=51	%
Metabolic causes	5	9.8
Pulmonary diseases	13	25.49
Cardiac failure	2	3.92
Neurologic compromise	24	47.05
Genetic disease	3	5.88
Post-surgical admission	4	7.84

Onset of VAP in the studied patients with nosocomial VAP infection

Most of studied patients [35 out of 51 (68.6%)], had late onset pneumonia that was after or on fifth day of intubation, while 16 out of 51 mechanical ventilated patients (31.4%) had early onset of VAP, as shown in figure 1.

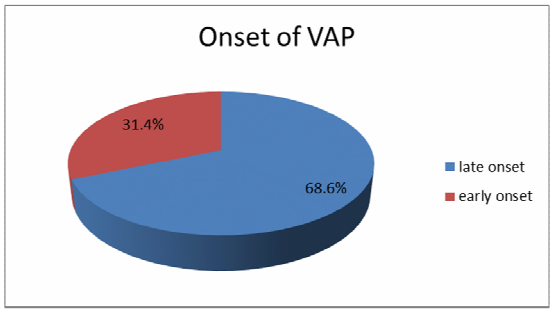

Fig. 1: Onset of VAP in the patients with nosocomial VAP infection.

Identification of isolated strains from endotracheal aspirates

Culture on Blood and MacConkey`s agar

All isolated (51) endotracheal aspirates specimens showed significant bacterial count ≥ 10^6 CFU/ml and all isolated bacteria were identified.

Gram stain

Gram negative bacteria were isolated from forty specimens 40(78.43%) while Gram positive bacteria were isolated eleven specimens 11 (21.56%).

Out of 40 Gram negative bacteria, 14(35%) were non lactose fermented bacteria and 26 (65%) were lactose fermented bacteria.

Out of 26 lactose fermented bacteria, 19 were *Klebsiella pneumoniae* which identified by morphology and biochemical tests.

K. pneumoniae grew on blood agar as non-hemolytic, grey, round, shiny and mucoid colonies, grew on macConkey's agar as lactose fermenting (LF) pink mucoid colonies, as shown in figure 2.

Fig. 2: *K. pneumoniae* on MacConkey`s agar.

K. pneumoniae was identified as Gram-negative, non-sporing rods, oxidase -ve, produced acidic butt and slant on TSI, +ve VP test, gave blue colony when cultured on citrate media and urease +ve, as shown in figure 3.

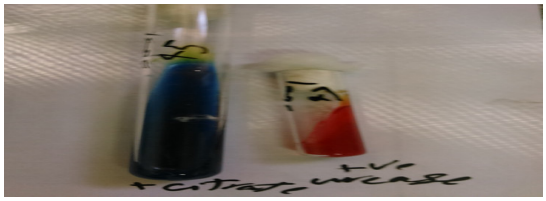

Fig. 3: *K. pneumoniae* on Simmon`s Citrate Agar and Christensen`s urea Agar.

Result of biotyping of *K. pneumoniae* isolates by API 20E kit

In our study, 19 conventionally identified *K. pneumoniae* isolates by biochemical test were tested using API 20E strips for further identification and biotyping of *K. pneumoniae* isolates.

The API 20E Index system identified 19 *Klebsiella* spp. isolates as *Klebsiella Pneumoniae* with two different analytical profile index numbers. The first biotype (B1) including 17 isolates with 5215773 code and second biotype (B2) including isolates with 1215773 code, as presented in figure 4 and table 3.

Result of antimicrobial susceptibility of *K. pneumoniae* isolates

Antibacterial susceptibility profile was performed to *K. pneumoniae* isolates by the Kirby Bauer disc diffusion method according to CLSI guidelines[6], in figure 5.

Analysis of the susceptibility patterns of *K. pneumoniae* isolates against 14 different

antibiotics showed that all tested strains were sensitive to colistin, and resistant to piperacillin, amoxacillin- clavulanate, cefazolin, cefipeme, cefoperazone, ceftazdime and ceftriaxone as presented in table 4.

Fig. 4: Biotyping of *K. pneumoniae* with API20E system.
(A): code 5215773, (B): code 1215773

Table 3: Biotyping of *K. pneumoniae* isolates by API 20E System.

Numerical profile	ONP	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
5215773	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
1215773	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Fig. 5: Antibacterial susceptibility profile of *K. pneumoniae* by the Kirby Bauer disc diffusion method.

Table 4: Antibacterial susceptibility patterns of *K. pneumoniae* isolates against 14 antimicrobial agents.

Isolates	PT	AMC	KZ	CPM	CEP	CA	CRO	IPM	MPM	CN	AK	LEVO	CT	TMP
K1	R	R	R	R	R	R	R	R	R	R	R	S	S	R
K2	R	R	R	R	R	R	R	I	R	R	R	S	S	R
K3	R	R	R	R	R	R	R	R	R	R	R	S	S	R
K4	R	R	R	R	R	R	R	R	R	R	R	R	S	R
K5	R	R	R	R	R	R	R	S	R	S	I	R	S	R
K6	R	R	R	R	R	R	R	R	R	R	R	R	S	R
K7	R	R	R	R	R	R	R	R	R	R	R	R	S	R
K8	R	R	R	R	R	R	R	R	R	I	R	S	S	I
K9	R	R	R	I	R	R	R	S	S	R	S	S	S	R
K10	R	R	R	R	R	R	R	I	R	R	R	S	S	R
K11	R	R	R	R	R	R	R	R	R	R	R	S	S	R
K12	R	R	R	R	R	R	R	R	R	R	R	R	S	S
K13	R	R	R	R	R	R	R	I	R	R	R	S	S	R
K14	R	R	R	R	R	R	R	R	R	R	R	S	S	R
K15	R	R	R	R	R	R	R	R	R	R	R	S	S	R
K16	R	R	R	R	R	R	R	R	R	R	R	S	S	R
K17	R	R	R	R	R	R	R	R	R	R	R	S	S	R
K18	R	R	R	R	R	R	R	I	R	R	R	S	S	R
K19	R	R	R	R	R	R	R	S	R	S	S	S	S	R

PT=piperacillin, AMC=amoxacillin- clavulanate, KZ=cefazolin, CPM=cefipeme, CEP=cefoperazone, CA=ceftazdime, CRO=ceftriaxone, IPM=imipenem, MPM=meropenem, CN=gentamicin, AK=amikacin, LEVO=levofloxacin, CT=colistin, TMP=trimethoprim-sulphamethoxazole, R=resistant, I=intermediate, S=sensitive.

Discussion

Ventilator-associated pneumonia (VAP) is defined as pneumonia taking place in patients receiving mechanical ventilation, 48 hours or more after the initiation of ventilation. It is considered the second most common nosocomial infection in pediatric intensive care units (PICUs)[7&8]. Such infection adversely affects patient's outcome and results in considerable morbidity and mortality. It also significantly increases medical costs by prolonging PICU and hospital stay[2]**.** The incidence of VAP in ICUs of Egyptian University Hospitals is about 2.5 times more than in the United States and worldwide[9].

In the current study, 51 patients in PICU diagnosed of nosocomial VAP infection were included. Out of 51 VAP infected male patients were 32 (62.7%) and females were 19 (37.3%). These results were similar to what previously reported[10].

In this study, the age of patients with nosocomial VAP infection ranged from one month to 14 years. Seventy (70%) of these infection occurred among infant patients aged <1 year. These results indicate the higher prevalence of nosocomial VAP infection among young age. In agreement with our results, what was reported by Hamid *et al*.[11] who stated that age less than 1-year-old is significantly associated with VAP in PICU.

Most of the patients admitted to the PICU had severe underlying diseases, such as Gillian bare syndrome, intracranial hemorrhage and convulsion, acute respiratory distress and chronic obstructive pulmonary disease. When considering the development of VAP in relation to the underlying condition, in our study it was seen that neurologic illness (47.05%) was the most common underlying condition among VAP infected patients. This result differs from the findings of other studies that reported that genetic syndromes and trauma are the most common risk factors for development of VAP in children[12&13]. This difference may be attributed to the fact that most of the pediatric patients admitted to PICU in Assiut Children's Hospital had neurologic diseases.

Most of the cases 35/51 (68.6%) had late onset VAP that developed after or on the fifth day of intubation. Our findings are in accordance to the study of Abd El-Kader[14] in Mansoura University who reported that long duration of pediatric ICU admission and long duration of mechanical ventilation (more than 12 days) are risk factors of development of VAP. Prolonged intubation might increase the incidence of VAP by facilitating colonization of potentially pathogenic organism in respiratory secretions or endotracheal tube biofilm formation[15].

Fifty-one endotracheal aspirates were collected from 51 VAP infected patients in PICU during the study period. Forty specimens (78.43%) contained Gram negative microorganism and 11 (21.56%) specimens contained Gram positive microorganism. The most isolated organism was *Klebsiella pneumoniae* isolated from 19 (37.25%) specimens which declares the importance of this pathogen among pediatrics VAP patients. These results are in parallel to a previous study conducted in Zagazig University Hospital where Badr *et al.*[16] reported that the Gram negative bacteria were isolated from the majority of VAP patients (68.6%), with *Klebsiella* organism predominating the positive culture (34.3%).

In previous Egyptian studies, the most common causative organisms of VAP were *Pseudomonas aeruginosa*[17&18], *Klebsiella pneumoniae*[19&20], *Enterobacter* spp.[14] and *Acinetobacter*[21]. In Europe and North America, *Staphylococcus aureus* was the most common pathogen[22&23], while in Saudi Arabia, *Pseudomonas aeruginosa* was the most common organism followed by *Staphylococcus aureus*[24]. The causes of VAP appear to differ even between different hospitals within the same city by the difference in microorganisms circulating in the environment[25].

Our results have shown that high level of resistance existed among clinical isolates against different classes of antibiotics, which is probably due to the frequent use of these antibiotics to treat pediatric patients. These results are in agreement with what was reported in a recent Egyptian study where all *K. pneumoniae* isolates were resistant to all tested beta lactams and beta lactams / beta lactamase inhibitor combination[26]. The current study suggested that PICU represent a well-established reservoir for MDR- *K. pneumoniae*

due to misuse of antibiotics among pediatrics patients. On the other hand, the resistance rate for imipenem was higher than that reported in previous studies[27&28]. The increased resistance of *K. pneumoniae* to impenem may be attributed to frequent use of meropenem in Assiut University PICU.

Our findings showed that the most effective antimicrobial agent against multi-drug resistant *K. pneumoniae* strains was colistin (100% sensitivity rate) followed by levofloxacin. Eida *et al*.[28] studied HAP in ICU and reported that the sensitivity rate of *K. pneumoniae* strains to levofloxacin was 44.8%. The high sensitivity rate of both colistin and levofloxacin may be due to that both drugs are not used in our PICU. Shawky *et al*.[26] observed that 86.2% of the *K. pneumoniae* isolates in their study were sensitive to colistin. This supports the evidence that colistin has increasingly become the best available therapeutic option for MDR- *K. pneumoniae* infections for pediatric patients.

Conclusion

Klebsiella pneumoniae is a common cause of nosocomial VAP infection in PICU at Assiut university children's hospital. Antimicrobial susceptibility testing showed that *K. pneumoniae* isolates were resistant to all commercial available antimicrobial agents that used in the PICU.

Recommendations

Strict implementation of a multidimensional infection control program which is essential for the reduction of VAP rate in PICUs in developing countries.

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نشرة العلوم الصيدلية
جامعة أسيوط

انتشار الكلبسيلا الرئوية المسببة للالتهاب الرئوى المرتبط بجهاز التنفس والمعزولة من وحدة العناية المركزة للأطفال فى مستشفى جامعة أسيوط

ايمان رمضان محمد[1] – حماده محمد محمد[1]– شرين أحمد عبدالرحمن[2]– شعبان احمد هاشم[2]

[1]قسم الميكروبيولوجى والمناعة ، كلية الصيدلة ، جامعة الأزهر ، فرع أسيوط ، مصر

[2]قسم الميكروبيولوجى والمناعة ، كلية الطب ، جامعة أسيوط ، أسيوط ، مصر

يعتبرالالتهاب الرئوى المرتبط بجهاز التنفس من عدوى المستشفيات الشائعة والتى تحدث للمرضى الذين هم على جهاز التنفس الآلى فى وحدات العناية المركزة للاطفال. تلعب الكلبسيلا الرئوية والمقاومة لكثير من المضادات الحيوية دورا هاما فى عدوى جهاز التنفس فى المرضى الحالات الحرجة.

تشتمل هذه الدراسة على ٥١ من مرضى الاطفال المصابين بالاتهاب الرئوى المرتبط بجهاز التنفس من وحدة العناية المركزة للأطفال فى مستشفى الاطفال جامعة أسيوط خلال فترة ١٢ شهر من مايو ٢٠١٤ الى مايو ٢٠١٥ ، باسخدام مزرعة افرازات القصبة الهوائية الكمى. لقد تم عزل الكلبسيلا الرئوية عن طريق زرعها على اطباق ماكونكى ودم اجار ثم تم تحضينها هوائيا عند درجة س ٣٥° لمدة ٢٤–٤٨ ساعة وتم التعرف عليها بالشكل الظاهرى والاختبارات الكيمياء الحيوية. تعتبر الكلبسيلا الرئوية أشهر بكتريا معزولة من وحدة العناية المركزة للاطفال ١٩(٣٧،٢٥٪) والمسببة للالتهاب الرئوى المرتبط بجهاز التنفس. اظهر اختبار الحساسية المضادة للميكروبات ان عزلات الكلبسيلا الرئوية كانت مقاومة لجميع المضادادت الحيوية المتوافرة تجاريا والتي تستخدم في وحدة العناية المركزة للاطفال.