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Prevalence and antimicrobial susceptibility of bacterial pathogens isolated from ventilator associated pneumonia (VAP) patients

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ABSTRACT

According to World Health Organization (WHO), lower respiratory tract infections are the third most common cause of death worldwide. These infections are mainly caused by multidrug-resistant (MDR) bacteria. Between 8 - 28% of patients receiving mechanical ventilation are affected by ventilator associated pneumonia (VAP).

The aim of current study was to characterize bacteria isolated from VAP patients and to evaluate the effectiveness of some antimicrobial agents. Clinical bacterial isolates were recovered from patients having pneumonia associated with mechanical ventilation from intensive care units of Zagazig University Hospital and identified using conventional microbiological methods. Antimicrobial susceptibility profile of these isolates against various antimicrobials was tested by the disk diffusion method.

A total of 233 isolates were recovered from 153 samples of endo-tracheal aspirates, compromising 203(87.1%) Gram negative and 30 (12.9%) Gram positive bacteria. The major isolates were *Klebsiella pneumoniae* (36.9%), *Escherichia coli* (21.04%), *Acinetobacter baumannii* (14.95%), *Pseudomonas aeruginosa* (14.16%) and *Staphylococcous aureus* (12.02%), coagulase negative *Staphylococcus spp* (0.86%), *Serratia mercescens* (0.43%). The isolates were highly resistant to antimicrobial agents. Two hundreds and twelve isolates (90.9%) were MDR and one hundred seventy two isolates (73.8%) were extensively drug resistant (XDR). Our study recommends that antimicrobial susceptibility should be performed for bacteria isolated from VAP patients before antimicrobial therapy to avoid emergence of MDR strains.

Key words: Ventilator associated pneumonia, antimicrobial susceptibility, multidrug resistance , extensively drug resistant .

INTRODUCTION

Pneumonia is defined as inflammation and consolidation of lung tissue due to an infectious agent **(Marrie, 1994; Jadavji *et al*., 1997).** The clinical symptoms and signs of pneumonia are nonspecific and variable **(Jadavji *et al*., 1997, Tan *et al*., 1998)**. Hospital-acquired pneumonia (HAP) is the second most common nosocomial infection after urinary tract infections. HAP is defined as pneumonia that occurs 48 hours or more after admission to hospital, which was not found at the time of admission **(Niederman, 1996; Tablan *et al*., 2003)**.

The incidence of HAP ranges from 5 to 15 cases per 1000 hospital admissions **(Louie *et al*., 1991; Everts *et al*., 2000; Sopena and Sabria, 2005).** In case of patients admitted to an intensive care unit (ICU), HAP occurs in up to 25% of patients **(Safdar *et al*., 2005)**, with approximately 70 -80% of episodes occurring during mechanical ventilation **(Esperatti *et al*., 2005).** In mechanically ventilated patients, local host defences are further reduced by the presence of an endotracheal tube which limits the effectiveness of the cough and produces mucociliary dysfunction. If the mucociliary clearance is slowed, respiratory tract mucus traps bacteria, which proliferate instead of

being removed, leading to colonization and infection **(Levine and Niederman, 1991).**

In developed countries, the concept HAP is well established,while in developing countries, the data are extremely sparse **(Allegranzi *et al.*, 2001)**. The pattern of pathogens causing HAP is characteristically different from that causing community-acquired pneumonia (CAP), with greater representation of Gram-negative bacteria such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and greater prevalence of multiple antibiotic resistance **(Murphy *et al.*, 1994; Iregbu and Anwaal, 2001)**.

The aim of current study was to characterize bacteria causing ventilator associated pneumonia in ICU in local area (Zagazig, Sharkia, Egypt) and to determine antimicrobial susceptibility status of bacteria isolated. In order to guide therapeutic options and to help in developing strategies to avoid spread of antimicrobial resistance .

MATERIALS and METHODS

Bacteria isolation and identification

A total of one hundred and fifty three clinical sputum specimens were collected from ventilator associated pneumonia patients of intensive care unit of Zagazig University Hospital over the period from January 2014 to April 2014 and from March 2015 to July2015 .The clinical samples were collected aseptically as endo-tracheal aspirates from ventilator associated pneumonia patients and transported to the microbiology laboratory. The specimens were cultured on nutrient agar, blood agar, Mac-Conkey agar and mannitol salt agar and incubated for 24 hours at 37°C. Isolated bacteria were identified using Gram staining and cultural characteristics and biochemical tests (**Collee *et al.*, 1996).** The isolates were subjected to following biochemical tests indole production test, methyl red/Voges-Proskauer test (MR/VP), citrate utilization test, hydrogen sulphide production and reaction on TSI agar, urease production test, motility test, catalase test , oxidase test, O/F test, pigment production test, growth on blood agar and coagulase test.

Antibiotic susceptibility testing

Bacterial isolates were tested for their *in vitro* susceptibility against antimicrobial agents by disk diffusion method according to Clinical and Laboratory Standards Institute **(CLSI, 2012)**. Disks of antibiotics were amikacin (AK,30µg), aztreonam (AZM, 30µg), azithromycin (AZM, 15µg), carbenicillin (CAR, 100µg), cefepime (FEP, 30µg), cefotaxime (CTX, 30µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30µg), chloramphenicol (C, 30µg), ciprofloxacin (CIP, 5µg) , clindamycin (DA, 10µg), colistin (CT, 25µg), erythromycin (E, 30µg), gentamicin (CN,10µg), imipenem (IPM, 10µg), levofloxacin (LEV, 5µg), linezolid (LZD, 30 µg), meropenem (MEM, 10µg), ofloxacin (OFX, 5µg), piperacillin (PRL, 100µg), piperacillin/tazobactam (TZP, 110µg), sulfamethoxazole/trimethoprim (SXT, 1.25/23.27µg), tetracycline (TE, 30µg). The recommended antibiotics were used for each type of isolates and results were interpreted according to **CLSI (2012).**

RESULTS

Isolation and identification

A total of 233 isolates were recovered from 153 clinical samples. Among the 233 isolates, 203 (87.1%) isolates were Gram negative bacteria and 30 (12.9%) isolates were Gram positive. Eighty three specimens (59.71%) showed poly-microbial infections, while 56 (40.29%) showed mono-microbial infection. One hundred seventy seven isolates (75.95%) were recovered from poly-microbial samples.

The bacterial isolates were identified according to **Koneman *et al.* (1997).** The most common microorganisms isolated were *Klebsiella pneumoniae* (36.9%), *Escherichia coli* (21.04%), *Acinetobacter baumannii* (14.95%), *Pseudomonas aeruginosa*

(14.16%) and *Staphylococcus aureus* (12.02%). Other microorganisms were coagulase negative *Staphylococcus* spp (0.86%) and *Serratia marcescens* (0.43%) as shown in **table (1).**

Table(1) Distribution of specimens and types of microorganisms isolated from VAP patients

Specimens	**No (%)**
No of specimens	**153(100%)**
Negative specimens	**14 (9.15%)**
Positive specimens	**139(90.85%)**
Poly-microbial specimens	**83/139(59.71%)**
Mono-microbial specimens	**56/139(40.29%)**
Microorganism	**No (%)**
Gram positive bacteria	**30 (12.9%)**
Staphylococcous aureus	**28(12.02%)**
Coagulase negative *Staphylococcous spp*	**2(0.86%)**
Gram negative bacteria	**203(87.01%)**
Klebsiella pneumoniae	**86(36.9%)**
Escherichia coli	**49(21.04%)**
Acinetobacter baumannii	**34(14.59%)**
Pseudomonas aeruginosa	**33(14.16%)**
Serratia mercescens	**1 (0.43%)**

Antibiotic susceptibility testing (AST)

K. pneumoniae isolates demonstrated resistance to cefepime (94.19%), ceftraixone (97.68%) and aztreonam (93.02%).The results are shown in (**Table 2**). All *K. pneumoniae* isolates (100%) were multi drug resistant (MDR) and seventy eight isolates (90.7%) were extensively drug resistant (XDR).

E. coli isolates showed high resistance to ceftraixone (97.96%) , aztreonam (95.92%) and cefepime (95.92%) **(Table 3)** .Fourty eight isolates(97.96%) were MDR and thirty one isolates(63.27%) were XDR.

Table(2) Antimicrobial susceptibility of *Klebsiella pneumoniae* isolates

Antimicrobials agents	Susceptibility of *K. pneumoniae* isolates to antimicrobials					
	(R)		(I)		(S)	
	No	%	No	%	No	%
CRO	84	97.68%	0	0	2	2.32%
FEP	81	94.19%	2	2.32%	3	3.49%
ATM	80	93.02%	0	0	6	6.98%
CIP	78	90.7%	6	6.98%	2	2.32%
TE	78	90.7%	4	4.65%	4	4.65%
SXT	70	81.4%	1	1.16%	15	17.44%
TZP	66	76.74%	1	1.16%	19	22.9%
CN	58	67.44%	2	2.32%	26	30.23%
AK	51	59.3%	2	2.32%	33	38.37
IMP	42	48.8%	5	5.8%	39	45.34%

(CN) gentamicin ; (AK) amikacin ; (IMP) imipenem; (TZP) piperacillin /tazobactam ; (CRO) ceftraixone ; (ATM) aztreonam ;(FEP) cefepime; (SXT) sulfamethoxazole /trimethoprin ;(cip) ciprofloxacin ; (TE) tetracycline. (No) Number of isolates; (R) resistant; (I) intermediate; (S) sensitive.

All *Acinetobacter baumannii* isolates were resistant to ceftazidime, piperacillin, ofloxacin, meropenem, imipenem, ceftraixone, cefepime and levofloxacin, while they were sensitivie to colistin **(Table 4).** All *A. baumannii* isolates (100%) were MDR and thirty one isolates (91.2%) were XDR.

Among *Pseudomonas aeruginosa*, all isolates demonstrated resistance to ceftraixone and carbenicillin **(Table 5).** Twenty eight isolates (84.84%) were MDR and twenty six isolates (78.78%) were XDR .

All *Staphylococcus* spp demonstrated resistance to ceftraixone, cefotaxime and methicillin and sensitivity to linezolid **(Table 6).** Sixteen isolates (53%) were MDR and six isolates (21.4%) were XDR (**Table 7).**

Table (3) Antimicrobial susceptibility of *E. coli* isolates

Antimicrobial agents	Susceptibility of *E. coli* isolates to antimicrobials					
	(R)		(I)		(S)	
	No	%	No	%	No	%
CRO	48	97.96%	0	0	1	2.04%
ATM	47	95.92%	1	2.04%	1	2.04%
FEP	47	95.92%	0	0	2	4.08%
TE	45	91.84%	2	4.08%	2	4.08%
CIP	44	89.8%	2	4.08%	3	6.12%
SXT	39	79.95%	1	2.04%	9	18.37%
CN	26	53.06%	0	0	23	46.94%
TZP	13	26.53%	11	22.45%	25	51.02%
AK	5	10.2%	10	20.4%	34	69.39%
IMP	2	4.08%	3	6.12%	44	89.8%

(AK) amikacin ; (CN) gentamicin ; (IMP) imipenem ; (TZP) piperacillin / tazobactam ; (CRO) ceftriaxone ; (FEP) cefepime ; (ATM) aztreonam ; (SXT)sulfamethoxazole /trimethoprim ; (CIP) ciprofloxacin ; (TE) tetracycline. (No) number of isolates; ; (R) resistant; (I) intermediate; (S) sensitive

Table (4) Antimicrobial susceptibility of *A. baumannii* isolates to tested antibiotic.

Antimicrobial agents	**Susceptibility of *A. baumannii* to antimicrobials**					
	(R)		(I)		(S)	
	No	%	No	%	No	%
CAZ	34	100%	0	0	0	0
CRO	34	100%	0	0	0	0
FEP	34	100%	0	0	0	0
IMP	34	100%	0	0	0	0
LEV	34	100%	0	0	0	0
MEM	34	100%	0	0	0	0
OFX	34	100%	0	0	0	0
PRL	34	100%	0	0	0	0
Ak	30	88.24%	2	5.89%	2	5.89%
SXT	23	67.65%	3	8.82%	8	23.53%
CN	17	50%	0	0	17	50%
CT	0	0	0	0	0	100%

(LEV) levofloxacin ; (SXT) sulfamethoxazole /trimethoprin ; (CN) gentamicin; (CRO) ceftraixone ;(FEP) cefepime ;(AK) amikacin ; (CAZ) ceftazidime ;(PRL)piperacillin ; (OFX) ofloxacin ; (MEM) meropenem ; (IMP) imipenem; (CT) colistin. (No) number of isolates, (R) resistant; (I) intermediate; (S) sensitive

Table(5) Antimicrobial susceptibility of *P. aeruginosa* isolates to tested antibiotics

Antimicrobial agents	Susceptibility of *P. aeruginosa* isolates to antimicrobials					
	(R)		(I)		(S)	
	No	%	No	%	No	%
CAR	33	100%	0	0	0	0
CRO	33	100%	0	0	0	0
CAZ	29	87.89%	0	0	4	12.11%
SXT	26	78.79%	4	12.11%	3	9.09%
PRL	26	78.79%	3	9.09%	4	12.11%
CIP	26	78.79%	1	3.03%	6	18.18%
CN	26	78.79%	0	0	7	21.21%
AK	24	77.73%	4	12.12%	5	15.15%
C	24	77.73%	4	12.12%	5	15.15%
IMP	17	51.51%	3	9.09%	13	38.23%
TZP	15	45.45%	8	24.24%	10	30.3%

(AK) amikacin;(C) cholramphenicol; (CN) gentamicin; ; (SXT) sulfamethoxazole /trimethoprin; (CRO) ceftraixone; (CAR) carbenicillin ; (CAZ) ceftazidime; (TZP) piperacillin /tazobactam ;(PRL)piperacillin; (IMP) imipenem; (cip) ciprofloxacin. (N) No of isolates*;* ; (R) resistant; (I) intermediate; (S) sensitive.

Table(6) Antimicrobial susceptibility of *Staphylococcus* spp isolates

Antimicrobial agents	Susceptibility of *Staph.spp* isolates to antimicrobials					
	(R)		(I)		(S)	
	No	%	No	%	No	%
ME	30	100%	0	0	0	0
CTX	21	70%	9	30%	0	0
CRO	21	70%	8	26.67%	1	3.33%
CIP	18	60%	2	6.67%	10	3.33%
AZM	18	60%	0	0	12	40%
E	18	60%	0	0	12	40%
CN	15	50%	0	0	15	50%
DA	11	36.67%	0	0	19	63.33%
C	11	36.67%	1	3.33%	18	60%
SXT	4	13.33%	2	6.67%	24	80%
VA	0	0	0	0	30	100%
LZD	0	0	0	0	30	100%

(SXT) sulfamethoxazole / trimethoprim ; (CRO) ceftriaxone ; (CTX) cefotaxime; (ME) methicillin; (VA) vancomycin ; (E) erythromycin ; (AZM) azithromycin ; (DA) clindamycin ; (CN) gentamicin; (LZD) linezolid; (CIP)ciprofloxacin; (C) chloramphenicol. (N) number of isolates; (R) resistant; (I) intermediate; (S) sensitive

Table(7) Multi drug resistant (MDR) and extensively drug resistant (XDR) isolates

Microrganisms	Total No of isolates	MDR No (%)	XDR No(%)
K. pneumoniae	86	86(100%)	78(90.7%)
E. coli	49	48(97.96%)	31(63.27%)
A. baummanii	34	34(100%)	31(91.2%)
P. aeruginosa	33	28(84.84%)	26(78.78%)
Staphylococcus spp	30	16(53.3%)	6(20%)
Total	233	212(90.99%)	172(73.8%)

Discussion

Ventilator-associated pneumonia (VAP) is the most frequent intensive care unit (ICU)-acquired infection, occurring in 9–24% of patients entubated for longer than 48 hr **(Morehead and Pinto, 2000)**. It is associated with increased morbidity, prolonged hospitalization, and increased healthcare costs **(Rello *et al*., 2002; Erbay *et al*., 2004).**

Klebsiella pneumoniae (36.9%) was the most frequently isolated microorganism in VAP patients followed by *Escherichia coli* (21.04%), *Acinetobacter baumannii* (14.95%), *Pseudomonas aeruginosa* (14.16%) and *Staphylococcus aureus* (12.02%). The results were similar to that of **Daef *et al*. (2016),** who reported that Gram negative isolates were the most pathogens in VAP and that *Klebsiella* spp ,was the predominant pathogen followed by *E. coli* then *Acinetobacter* spp.

While **Seweilam (2003)** reported *K. pneumoniae* (30.9%) as the most frequently isolated microorganism, the other microorganisms were found in a rate of 22.5% *P. aeruginosa*, 21.2% *Staph. aureus*, 12.8% *E.coli*, 9.8% *Proteus* spp and 2.8% *Citrobacter* spp. This variation in the type and percentage of etiological agent could be attributed to patients, units, hospitals or countries. The main epidemiological patterns may not only vary from unit to unit, but also in a given unit over the course of time and this is true for their associated susceptibility patterns **(Rello *et al*., 1993).** .

Acinetobacter baumannii demonstrated frequency (14.95%) within isolates. According to **Costa *et al.* (2001), Santucci *et al.* (2003)** and **Medina *et al.* (2007)** *A. baumannii* in patients in ICU is frequent and represented 14% up to 37% that was in agreement with our result. **Daef *et al.* (2016)** reported that *A. baumannii* represented by only (5.6%) which disagreed with shown results.

Pseudomonas spp were isolated at rate (14.6%). This was in accordance with **Tayel (2009)** who reported that frequency of *Pseudomonas* spp (14.2%). On the other hand **Daef *et al.*, (2016)** reported frequency of *Pseudomonas* spp was only (2.74%).

Staphylococcus aureus demonstrated frequency (12.02%). This result was in accordance to **Galal *et al.*(2016)** who reported frequency of *Staphylococcus aureus* (14.4%).

Regarding resistance profiles our results noted that the most common causative organisms are mostly multi drug resistant (MDR) pathogens and non MDR pathogens are less likely as a cause. These was in agreement with **Loscalzo *et al*. (2011).**

The present study showed that gram negative bacteria had high resistance to many groups of antimicrobials as penicillins, cephalosporins and quinolones (50 to 100%). In agreement with this, **Ashour and ElSharif (2009)** reported high resistance to many groups of antibiotics in Egypt.

All Gram negative isolated pathogens had high resistance to cefotaxime, ceftazidime and ceftriaxone. These results suggest a high prevalence of extended spectrum B lactamase producing strains. Similar results were found by **Mukhopadhya *et al*. (2010**) as all the enterobacterial isolates in their study were ESBL producing.

All *A. baumanii* isolates were MDR while thirty one (91.2%) isolates were XDR. A study of **Varun *et al*. (2012)** reported that *Acinetobacter baumannii* isolates (100%) were multidrug resistant (MDR) that is, resistant to three or more class of antibiotics .The high rates of antimicrobial resistance identified in the present study is similar to that of **Daef and Elsherbiny (2012)**. They reported gram negative bacteria with high resistance (50 to 100%) to many groups of antimicrobials, as penicillins, cephalosporins, quinolones and aminoglycosides.

Resistance of gram positive organisms to macrolides (azithromycin and erythromycin) was 50 to 100%. This was compatable with what reported by **Ahmed *et al.* (2011)** that the resistance of gram positive bacteria to macrolides were 64.3 and 66.4%

Gram-positive isolates in the present study were highly resistant to penicillins and cephalosporins. These antibiotics are commonly prescribed empirically in the ICUs. Lower resistance was detected to chloramphenicol which may reflect the reduction in useage of it. This agreed with what reported by **Daef *et al*., (2016).**

In conclusion VAP is a common and serious hospital acquired infection. For better management of VAP, our study recommends that periodic epidemiological investigation of the most encountered pathogens causing VAP and antimicrobial susceptibility test should be performed before antimicrobial therapy to help choosing the appropriate antibiotics and avoid emergence of MDR strains.

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انتشار وحساسيه البكتريا الممرضه للمضادات الميكروبيه المعزوله من مرضي الالتهاب الرئوي المصاحب لجهاز التنفس الصناعي

فتحي سري – همت كمال - أميرة الجنايني- فاطمه الزهراء احمد

طبقا لمنظمه الصحة العالمية ، تعتبر عدوي الجهاز التنفسي السفلي هي ثالث اكثر الاسباب شيوعا التي تؤدي للموت عبر العالم . تحدث هذه العدوي بكتريا شديد المقاومه للمضادات الحيويه. فقد وجد ان من بين ٨ الي ٢٨% من المرضي الموضوعين علي جهاز تنفس الصناعي يصابون بالالتهاب الرئوي المصاحب للجهاز التنفس الصناعي.

استهدفت الدراسة الحالية إلي التعرف علي البكتريا المساهمة في إلتهاب الرئوي الناتج عن استعمال اجهزه التنفس الصناعي و دراسه مقاومتها للمضادات الميكروبيه شائعه الاستعمال .

تم اجراء هذه الدراسة علي ١٥٣ عينة نضح من مرضي إلتهاب رئوي ناتج من جهاز التنفس الصناعي بالعنايه المركزه بمستشفي جامعة الزقازيق خلال الفترة من يناير ٢٠١٤إلي ابريل٢٠١٤ ثم من مارس ٢٠١٥ الي يوليو ٢٠١٥.

انتجت العينات ٢٣٣ عزلة وتبين أنها تمثلت في كليبسيلا بنسبة (٣٦,٩%) و ايشريشيا كولاي بنسبة (٢١,٠٤%) واسنتوباكتر بومنياي بنسبة (١٤,٩٥%). كما تم عزل بكتيريا سودوموناس ايروجينوزابنسبة (١٤,١٦%) و ستافيلوكوكس أوريس بنسبة (١٢,٠٢%) وستافيلوكوكس ايبيدرميد بنسبة (٠,٨٦ %) و سيراشيا مرسيسنز بنسبه (٠,٤٣ %).

تم اختبار حساسية البكتريا المعزولة للمضادات الحيوية المختلفة المستخدمة و الموجودة في السوق المصري. أظهرت الدراسه الحاليه ان هناك اربع عشر عينه من الكليبسيلا لديهم مقاومه لجميع المضادات الحيويه المستخدمه . كما وجدت لكليبسيلا و الايشريشيا مقاومه عاليه الي الي سيفترايكسون و السيفيبيم بنسب (٩٧% و٩٤%) كما أظهرت مقاومة اقل بنسبة(٤٨,٨%) للايمبينم . بينماأظهرت عزلات الاسنيتوبكتر حساسية بنسبة (١٠٠%) للكوليستين سلفات و مقاومه شديده بنسبه (١٠٠%) لكل من سيفترايكسون و السيفتازديم و سيفيبيم و الاوفلوكساسين وليفوفلوكساسين والاميبنيم والميروبينم. علي الجانب الآخر أظهرت عزلات السيدوموناس ايروجينوزا مقاومه شديده ايضا بنسبة (١٠٠%) لكل من الكاربنسيلين و السيفترايكسون .

و من هذه الدراسة تبين ضروره القيام بالمزرعه البكتيريه قبل اعطاء المضادات الحيويه و ذلك لتنوع البكتيريا المسببه للالتهاب الرئوي المصاحب لجهاز التنفس الصناعي و اختلاف مقاومتها للمضادات الحيويه المستخدمه.