

УДК 579.61

DOI: 10.31040/2222-8349-2019-0-1-42-46

## ISOLATION AND IDENTIFICATION OF MICROORGANISMS ASSOCIATED WITH RESPIRATORY TRACT INFECTIONS FROM PATIENTS IN EGYPT

© G.O.O. Hassan, M.E.A. Mosa, M.W.A. Ibrahim, N.S. Karamova

The study was carried out to isolate and identify the common microorganisms associated with respiratory tract infections (RTIs) among patients admitted to two hospitals in Cairo, Egypt, from May to September 2014. Clinical materials (sputum, endotracheal tube, nasal and laryngeal swabs) were obtained from patients with respiratory tract infections symptoms from different age groups, in Kasr El-Aini and El-Rahma Masr El Gedida hospitals, Egypt. Ninety (90) microorganisms were isolated from one hundred (100) clinical samples. Clinical materials taken from fifty-five patients (55, 84.6%) out of sixty-five (65) examined contained various microorganisms, whereas clinical specimens from ten patients (10, 15.4%) were without any microorganisms. Male patients 38 (69%) had more microorganisms than female patients 17 (31%). Different methods for identification of isolated microorganisms: cultural characterization, conventional biochemical methods, the Analytical Profile Index test (API-10S, API-20 strep test systems) and MALDI-TOF Mass Spectrometry methods were used. The microorganisms isolated from patients with respiratory tract infections were 5 Gram-negative bacteria: *Klebsiella pneumoniae* ( $n = 20$ , 22.2%), *Escherichia coli* ( $n = 8$ , 8.9%), *Pseudomonas aeruginosa* ( $n = 7$ , 7.8%), *Acinetobacter baumannii* ( $n = 7$ , 7.8%) and *Enterobacter cloacae* ( $n = 1$ , 1.1%); 5 Gram-positive bacteria: *Staphylococcus epidermidis* ( $n = 15$ , 16.7%), *Staphylococcus aureus* ( $n = 7$ , 7.8%), *Enterococcus faecalis* ( $n = 2$ , 2.2%), *Corynebacterium striatum* ( $n = 1$ , 1.1%) and *Bacillus subtilis* ( $n = 3$ , 3.3%) and fungi: *Candida tropicalis* ( $n = 19$ , 21.1%). Thus, *Klebsiella pneumoniae*, *Candida tropicalis* and *Staphylococcus epidermidis* were the most predominant microorganisms isolated from the clinical materials obtained from the patients with symptoms of RTIs.

Key words: Respiratory tract infections, microorganisms, isolation, identification.

**Introduction:** The respiratory tract can be classified into two parts: the upper respiratory tract (URT) consists of a nose, a nasal cavity, sinuses, pharynx, epiglottis, larynx and the lower respiratory tract (LRT) consists of trachea, bronchi, and lungs (Perkin, 2003). The upper respiratory tract infections are more common, but lower respiratory tract infections are generally more severe. RTIs represent the most common acute illness evaluated in the outpatient setting. Moreover, acute RTIs, in particular pneumonia, remain one of the most important causes of death in both adults and children (over 50 million deaths in the entire world). With approximately 1.4–1.8 million fatal cases per year in children under the age of five, pneumonia causes

more fatalities than AIDS, malaria, and measles combined (Bosch *et al.*, 2013). The most of RTIs are viral; however, bacterial infections are also responsible for considerable morbidity and have potential for life-threatening consequences (Morris, 2007). The correct identification of the etiological agents is important for proper treatment, in particular for appropriate prescribing of antibiotics. The aim of this study was the isolation and identification of microorganisms associated with the respiratory tract infections among patients of two hospitals in Cairo, Egypt.

**Materials and methods: Collection area.** One hundred clinical material samples from upper

HASSAN Gamal Osman Osman, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia and Desert Research Center, Medicinal and Aromatic Plants Department, Cairo, Egypt, e-mail: gamal\_micro84@yahoo.com

MOSA Mohamed El-Sayed Abd El-Maksoud, Central Public Health Laboratory, Ministry of Health, Cairo, Egypt, e-mail: gamal\_micro84@yahoo.com

IBRAHIM Mai Wisam Ahmed, Central Public Health Laboratory, Ministry of Health, Cairo, Egypt, e-mail: gamal\_micro84@yahoo.com

KARAMOVA Nazira Sunagatovna, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia, e-mail: gamal\_micro84@yahoo.com

and lower respiratory tract of patients admitted to the Kasr El-Aini and El-Rahma Masr El Gedida hospitals (Egypt) were collected in the period of May to September 2014. **Clinical materials:** sputum, endotracheal tube (ETT), nasal swab and laryngeal swab from 65 patients of both genders and different age groups with RTIs symptoms. **Sample inoculation and purification.** The clinical material samples were inoculated onto the surface of Petri dishes which contained different media (Blood, Chocolate, CLED and MacConkey agar) using sterilized loop for the isolation of microorganisms. All plates were incubated at 37°C for 18–24 hours while the chocolate agar plates were incubated at 37°C under anaerobic conditions for 18–24 hours. Then purified colonies were obtained by repeated streaking of the single colony on fresh nutrient agar plates. Pure culture colonies were sub-cultured on agar medium. Colonies parameters: size, shape, texture, odour, pigmentation and hemolysis were observed. Gram staining to differentiate the bacterial isolates into Gram-positive and Gram-negative groups was carried out (Cheesbrough, 2005). **Identification of bacteria:** The isolated microorganisms were differentiated and identified by various conventional biochemical methods (biochemical analysis) such as triple sugar iron (TSI), lysine decarboxylase (LDC), (MIO) Motility Indole Ornithine testes, citrate utilization, urease, oxidase, coagulase testes (Cheesbrough, 2005). Catalase activity analysis to distinguish among *Streptococcus* and *Staphylococcus* species (Cappuccino, 2002), a deoxyribonuclease (DNase) activity test to differentiate between *Staphylococcus aureus* and *Staphylococcus epidermidis* and bile-esculin test to distinguish between *Streptococcus* and *Enterococcus* were performed. Then Analytical profile index (API 10S, API-20 strep test systems) and MALDI-TOF Mass Spectrometry method were used as well.

**Results and Discussion:** Different microorganisms were isolated from clinical materials obtained from 55 patients while we did not find any microorganisms in clinical specimens taken from the other 10 patients. Out of the 90 isolated microorganisms, 65 (72.2%) were from males while 25 (27.8%) were from females (Table 1). From the results obtained it appears that differences in anatomy, lifestyle, behavior and etc. could have a significant role in the development of RTIs in males and females. It is similar with the data of Panda *et al.*, (2012) who reported that out of the 101 isolated microorganisms, 64 (63.4%) were from males and

37 (36.6%) were from females. Basing on the analysis of data extracted from 84 relevant studies Falagas *et al.* emphasize that males are more susceptible than females to most types of RTIs in all age groups. They also note that the sex hormones could contribute to the reported sex differences in the incidence of RTIs for their role in the regulation of the immune system (Falagas *et al.*, 2007).

A total 90 isolates of microorganisms have been classified by Gram staining. Gram-negative organisms accounted for 43 (47.8%) and Gram-positive accounted for 28 (31.1%) while the number of fungi was 19 (21.1%) out of all isolated microorganisms. Gram-negative bacteria were classified by biochemical methods into 5 groups which have the same biochemical characteristics (Tables 2, 4, 5). Gram-positive bacteria were grouped into 5 different groups which have similar criteria (Tables 3, 5)

The predominant isolates of Gram-negative bacteria were identified as *Klebsiella pneumoniae* (22.2 %), *Escherichia coli* (8.9%) while among the isolated Gram-positive bacteria *Staphylococcus epidermidis* (16.7%) and *Staphylococcus aureus* (7.8%) were the predominant microorganisms. The high percentage of *K. pneumoniae* infection could be explained by the fact that many of patients have been taking inappropriate antibiotics. It is known that *K. pneumoniae* is naturally resistant to aminopenicillins (i.e. ampicillin or amoxicillin), an antibiotic that is commonly used as the first line antibiotic therapy in RTI in many developing countries. The problem is complicated by the fact that the etiological agents of RTIs must be more precisely defined before treatment. Notable, many studies carried out in different countries demonstrate that Gram-negative bacteria including *K. pneumoniae* species were the common microorganisms isolated from patients with RTIs. The ratio of *Klebsiella pneumoniae* (22.2%) found in this study similar to the earlier data obtained by other authors, for example, Taura *et al.*, (2013) in Nigeria and in other countries such as Cameroun, South Africa, China, Japan and Turkey as well. Ratio of *Staphylococcus aureus* (7.8%), *Escherichia coli* (8.9%) and *Staphylococcus aureus* (7.8%) corresponded to the findings of Khan *et al* (2015). On the other hand, the results of our study disagree with the data reported in work of Richard *et al.*, (2000), where *Staphylococcus aureus* was the most frequently identified isolate (17%) followed by *Pseudomonas aeruginosa* (15.66%), *Enterobacter* species (10.9%) and *Klebsiella pneumoniae* (7.0%).

**Conclusions:** The respiratory tract infections are among the most common and important problems in Egypt and most African countries. The control of respiratory tract infections is frequently difficult because of the problem connected with the identification of the etiological agents and frequent cases of self-medication. Identification of microorganisms associated with RTIs by more than one

method is very important in order to determine the appropriate therapy based on the testing of antibiotic sensitivity for the true causative agents.

Table 6 summarize the distribution of different species among the total 90 microbial isolates from clinical materials taken from 55 patients with RTI symptoms.

Table 1

*Occurrence of microorganisms isolated from patients with RTIs based on gender*

Gender	Number of patients examined	Number of patients with microorganisms	Number of patients without microorganisms
Male	43 (66.2%)	38 (69%)	5 (50%)
Female	22 (33.8%)	17 (31%)	5 (50%)
Total	65 (100%)	55 (84.6%)	10 (15.4%)

Table 2

*Characteristics of isolated Gram-negative bacteria by conventional biochemical methods*

Characteristics	Groups of isolates				
	GN1	GN2	GN3	GN4	GN5
Cellshape	Rod	Rod	Rod	Rod	Rod
TSI	K/N	A/A	A/A	A/A	K/N
LDC	-	-	+	+	-
MIO	---	+++	+++	---	---
Citrate	+	+	-	+	+
Urea	-	-	-	+	-
Oxidase	+	-	-	-	-

+ indicates positive reaction results; – indicates negative reaction results, K: Alkaline; A: acid; N: negative.

Table 3

*Characteristic of isolated Gram-positive bacteria by biochemical methods*

Characteristics	Groups of isolates				
	GP7	GP6	GP8	GP9	GP10
Sporeformation	-	-	-	-	+
Cell shape	spherical	spherical	spherical	Rod	Rod
Catalase	+	+	-	+	+
DNase	+	-	-	N.T	N.T
Bile-esculin	N.T		+	N.T	N.T
Coagulase	+	-	-	N.T	N.T

NT: no test; + indicates positive reaction results; – indicates negative reaction results.

Table 4

## Identification of isolated Gram-negative bacteria with API 10s test

Character	GN1	GN2	GN3	GN4	GN5
ONPG	-	+	+	+	-
GLU	+	+	+	+	+
ARA	+	+	+	+	+
LDC	-	-	+	+	-
ODC	-	+	-	-	-
CIT	+	+	-	+	+
H <sub>2</sub> S	-	-	-	-	-
URE	-	-	-	+	-
TDA	-	-	-	-	-
IND	-	-	-	-	-
OX	+	-	-	-	-
NO <sub>2</sub>	-	+	+	+	-

ONPG: o-nitrophenyl-b-D-galactopyranoside, GLU: glucose, ARA: arabinose, LDC: lysine decarboxylase, ODC: ornithine decarboxylase, CIT: citrate, H<sub>2</sub>S: hydrogen sulfide, URE: urease, TDA: tryptophan deaminase, IND: indole, OX: oxidase, NO<sub>2</sub>: nitrite.

Table 5

## Identification of microbial isolates by MALDI-TOF mass spectrometry

Analytic ID	Organism	Score value
GO1	<i>Pseudomonas aeruginosa</i>	2.371
GO2	<i>Enterobacter cloacae</i>	2.208
GO3	<i>Escherichia coli</i>	2.452
GO4	<i>Klebsiella pneumoniae</i>	2.509
GO5	<i>Acinetobacter baumannii</i>	2.329
GO6	<i>Staphylococcus epidermidis</i>	2.158
GO7	<i>Staphylococcus aureus</i>	2.42
GO8	<i>Enterococcus faecalis</i>	2.363
GO9	<i>Corynebacterium striatum</i>	2.329
GO10	<i>Bacillus subtilis</i>	2.065
GO11	<i>Candida tropicalis</i>	2.05

Table 6

## Distribution of total microorganisms isolated from the patients with respiratory tract infections

No	Species	Numbers of isolates	Percentage
1	<i>Klebsiella pneumoniae</i>	20	22.2%
2	<i>Candida tropicalis</i>	19	21.1%
3	<i>Staphylococcus epidermidis</i>	15	16.7%
4	<i>Escherichia coli</i>	8	8.9%
5	<i>Acinetobacter baumannii</i>	7	7.8%
6	<i>Staphylococcus aureus</i>	7	7.8%
7	<i>Pseudomonas aeruginosa</i>	7	7.8%
8	<i>Bacillus subtilis</i>	3	3.3%
9	<i>Enterococcus faecalis</i>	2	2.2%
10	<i>Corynebacterium striatum</i>	1	1.1%
11	<i>Enterobacter cloacae</i>	1	1.1%
Total		90	100

References

1. Bosch A., Biesbroek G., Trzcinski K., Sanders E. and Bogaert D. Viral and bacterial interactions in the upper respiratory tract // PLoS Pathog., 2013. № 1. P. 1–12.
2. Cappuccino S. Microbiology, a laboratory manual // 6th ed. Benjamin Camings., 2002. P. 149–155.
3. Cheesbrough M. District Laboratory Practice in Tropical Countries part 2 // Cambridge University Press, UK., 2005. P. 105–194.
4. Falagas M.E., Mourtzoukou E.G., Vardakas K.Z. Sex differences in the incidence and severity of respiratory tract infections // Respir Med., 2007. № 9. P. 1845–1863.
5. Khan S., Priti S., and Ankit S. Bacteria etiological agents causing lower respiratory tract infections and their resistance patterns // Iran. Biomed. J., 2015. № 4. P. 240–246.
6. Morris D. P. Bacterial biofilm in upper respiratory tract infections // Current Infectious Disease Reports., 2007. № 3. P. 186–192.
7. Panda S. B., Nadini P., Ramani T. V. Lower respiratory tract infection-bacteriological profile and antibiogram pattern // Int. J. Cur. Res., 2012. № 21. P. 149–155.
8. Perkins M. Respiration power point presentation // Biology 182 course Handout / Orange Coast College, Customers, CA. 2003.
9. Richards M.J.R., Edwards D.H., Culver R.P. Gaynes and the national nosocomial infections surveillance system // Nosocomial infections in combined medical surgical intensive care units in the United States. Infect. Control Hosp. Epidemiol., 2000. № 21. P. 510–515.
10. Taura D.W., Hassan A., Yayo A.M. and Takalmawa H. Bacterial isolates of the respiratory tract infection and their current sensitivity pattern among patients attending Aminu Kano Teaching Hospital Kano-Nigeria // International Research Journal of Microbiology (IRJM), 2013. № 9. P. 226–231.

**ВЫДЕЛЕНИЕ И ОПРЕДЕЛЕНИЕ МИКРООРГАНИЗМОВ, ВЫЗЫВАЮЩИХ  
РЕСПИРАТОРНЫЕ ИНФЕКЦИИ У БОЛЬНЫХ ИЗ ЕГИПТА**

© Г.О.О. Хассан<sup>1, 2</sup>, М.Э.А. Моса<sup>3</sup>, М.В.А. Ибрахим<sup>3</sup>, Н.С. Карамова<sup>1</sup>

<sup>1</sup> Институт фундаментальной медицины, Казанский федеральный университет,  
Казань, Российская Федерация

<sup>2</sup> Исследовательский центр изучения пустынь,  
Отдел лекарственных и ароматических растений. Каир, Египет

<sup>3</sup> Центральная лаборатория общественного здравоохранения,  
Министерство здравоохранения, Каир, Египет

Проведено исследование с целью выделения и определения наиболее распространенных микроорганизмов, вызывающих респираторные инфекции, у больных, проходивших лечение в двух каирских клиниках (Египет) с мая по сентябрь 2014 г. Клинические материалы (мокрота, содержимое эндотрахеальной трубки, мазок из зева и носа) были взяты у больных различных возрастных групп с симптомами респираторных инфекций, проходивших лечение в клиниках Каср-Эль-Айни и Эль-Рахма Маср-Эль-Гедида в Каире. Из ста клинических образцов было выделено девяносто микроорганизмов. Клинические материалы, взятые у пятидесяти пяти (84.6%) из шестидесяти пяти осмотренных больных, содержали различные микроорганизмы, тогда как в клинических образцах, взятых у десяти больных (15.4%), таковых не обнаружено. У пациентов-мужчин (69%) микроорганизмы встречались чаще, чем у женщин (31%). Были использованы различные методы определения выделенных микроорганизмов: характеристика культуральных свойств, стандартные биохимические методы, тест на определение аналитического индекса профилей (тесты на стрептококк API-10S, API-20) и методы масс-спектрометрии MALDI-TOF. Микроорганизмы, выделенные у больных с респираторными инфекциями, включали пять грамположительных бактерий: *Klebsiella pneumoniae* ( $n = 20$ , 22.2%), *Escherichia coli* ( $n = 8$ , 8.9%), *Pseudomonas aeruginosa* ( $n = 7$ , 7.8%), *Acinetobacter baumannii* ( $n = 7$ , 7.8%) и *Enterobacter cloacae* ( $n = 1$ , 1.1%); пять грамотрицательных бактерий: *Staphylococcus epidermidis* ( $n = 15$ , 16.7 %), *Staphylococcus aureus* ( $n = 7$ , 7.8%), *Enterococcus faecalis* ( $n = 2$ , 2.2%), *Corynebacterium striatum* ( $n = 1$ , 1.1 %) и *Bacillus subtilis* ( $n = 3$ , 3.3%), а также грибок *Candida tropicalis* ( $n = 19$ , 21.1%). Доминирующими микроорганизмами в клинических материалах, взятых у больных с симптомами респираторных инфекций, были, таким образом, *Klebsiella pneumoniae*, *Candida tropicalis* и *Staphylococcus epidermidis*.

Ключевые слова: респираторные инфекции, микроорганизмы, выделение, определение.