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Molecular analysis of *Klebsiella pneumoniae* ESBL (+) isolated from patients hospitalized in the National Tuberculosis and Lung Diseases Research Institute at the Intensive Care Unit

Analiza molekularna szczepów *Klebsiella pneumoniae* ESBL (+) izolowanych od chorych hospitalizowanych w Instytucie Gruźlicy i Chorób Płuc w Oddziale Intensywnej Terapii

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Słowa kluczowe

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Summary

Introduction. Bacillus from *Enterobacteriaceae* ESBL (+) including *Klebsiella pneumoniae* are the most common pathogen of digestive tract. Patients colonized with hospital-acquired infections fall sick on hospital-pneumonia eight times more often than patients with their own bacterial flora. ESBL (+) strains determine serious threat with limited ways of treatment. Transfer of microorganisms in hospitals is followed by nursery and medicinal operations (thermometers, hands of staff). When the epidemic outbreak occurs in the hospital the procedure of contact isolation and microbiological monitoring must be conducted.

Aim. The aim of the study was to analyze of genetic relationship between *Klebsiella pneumoniae* ESBL (+) strains isolated from patients hospitalized in the National Tuberculosis and Lung Diseases Research Institute (NTLDRI).

Material and methods. Material of analysis were 64 strains of *Klebsiella pneumoniae* ESBL (+) isolated from 27 patients hospitalized on ICU. The phenotypic qualities were taken to the analysis and also the analysis of genetic relationship was conducted. The identification of species and the drug resistance phenotype were determined by the automatic system Vitek 2 Compact.

Results. All of the isolated strains belonged to the *Klebsiella pneumoniae* species and presented the mechanism of ESBL (+) resistance type. The analysis of genetic relationship of 7 strains indicated the existence of the same molecular pattern for 5 of the isolated strains. Strains which belonged to the same genotype were isolated from patients hospitalized on the same units. Therefore it can be assumed that the transmission of infection occurred in the area of patient's residence.

Conclusions. Molecular studies together with an indication of drug resistance should be the duty of laboratories and epidemiological investigation conducted in the supervision of nosocomial infections.

Streszczenie

Wstęp. Jednym z elementów kontroli zakażeń szpitalnych jest nadzór mikrobiologiczny, którego głównym celem jest analiza czynników etiologicznych zakażenia i określenie wzorów lekooporności na leki. Transmisja zakażenia w warunkach szpitalnych następuje podczas czynności pielęgnacyjnych i leczniczych chorego. W przypadku identyfikacji ogniska epidemicznego należy wprowadzić procedurę izolacji kontaktowej oraz monitorować wyniki badań. Postępowanie to jest konieczne w celu eliminacji lub ograniczenia ryzyka rozprzestrzeniania się zakażenia szpitalnego.

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Cel pracy. Celem pracy była analiza pokrewieństwa genetycznego szczepów *Klebsiella pneumoniae* ESBL (+) β -laktamazy o rozszerzonym spektrum substratowym (ang. *extended-spectrum β -lactamases* – ESBL) wyizolowanych od chorych hospitalizowanych w Instytucie Gruźlicy i Chorób Płuc (IGiChP). Analizę molekularną przeprowadzono w związku z podejrzeniem wystąpienia ogniska epidemicznego w Oddziałach Intensywnej Terapii.

Materiał i metody. Materiał do badań stanowiły 64 szczepy *Klebsiella pneumoniae* ESBL (+) wyhodowane od 27 chorych hospitalizowanych w Oddziale Intensywnej Terapii (OIT) i Intensywnej Terapii Pneumonologiczno-Kardiologicznej (OR) w okresie sześciu miesięcy w 2013 roku. W trakcie analizy zaobserwowano wzrost izolacji szczepów *Klebsiella pneumoniae* ESBL (+) w okresie kwiecień-maj. W celu stwierdzenia czy w ww. okresie w oddziałach OIT i OR wystąpiło ognisko epidemiczne, analizie poddano 7 szczepów *Klebsiella pneumoniae* ESBL (+) o takich samych cechach fenotypowych oraz przeprowadzono molekularną analizę wzorów DNA. Identyfikację wyhodowanych szczepów oraz wrażliwość na antybiotyki wykonano w automatycznym systemie Vitek 2 Compact.

Wyniki. Wszystkie wyizolowane szczepy należały do gatunku *Klebsiella pneumoniae* i wykazywały mechanizm oporności typu ESBL (+). Spośród 7 szczepów ten sam wzór molekularny posiadało 5 szczepów. Szczepy należące do tego samego genotypu pochodziły od chorych z dwóch sąsiadujących ze sobą Oddziałów Intensywnej Terapii. Do transmisji zakażenia doszło w obrębie miejsca pobytu chorego.

Wnioski. Stwierdzono jednakowy fenotyp i wzór molekularny szczepów *Klebsiella pneumoniae* wyizolowanych od 5 chorych w Oddziale Intensywnej Terapii i Intensywnej Terapii Pneumonologiczno-Kardiologicznej (OR), który świadczy o transmisji zakażenia pomiędzy chorymi.

Badania molekularne wraz z określeniem oporności na leki powinny być obowiązkiem laboratoriów w prowadzonym dochodzeniu epidemiologicznym oraz w nadzorze nad zakażeniami szpitalnymi.

INTRODUCTION

Several species of Gram-negative bacteria of the *Enterobacteriaceae* family are widespread in the environment and are also found in the digestive tract of animals and man. Some of them, particularly the *Escherichia coli* and *Klebsiella pneumoniae* species are the predominant pathogens in inpatient and outpatient treatment infections (1, 2).

Klebsiella pneumoniae rods cause human infections of varying severity – from asymptomatic intestinal colonization, colonization of the urinary and respiratory tracts to pneumonia, sepsis and fatal meningitis. *Klebsiella pneumoniae* infections are mostly caused by strains resistant to β -lactam antibiotics, with β -lactamase production with broad substrate ESBL (+) (1-3).

In recent years, *Enterobacteriaceae* ESBL (+) rods (extended-spectrum β -lactamase), including *Klebsiella pneumoniae* ESBL (+), have become one of the most important pathogens responsible for nosocomial infections. These are mainly patients with risk groups residing in intensive care units (4). The specificity of these wards is the use of specialized techniques of healing involving treatment using invasive monitoring and supporting the mechanical action of failing organs or systems. ICU patients are a special group of patients, because their long-lasting hospitalization can lead to infections with “hospital bacteria” which are a threat to the health or life of the patients (2, 4).

An important element of hospital infection control is microbial surveillance whose main objective is to analyze the etiological factors of infection and their resistance to antibiotics (5). In hospitals transfer of microorganisms occurs during operations and medical care (thermometers, staff hands). In the event of an epidemic outbreak in a hospital,

it is necessary to introduce contact isolation procedures and analyze the results of microbiological tests. This practice is the basis for the introduction of procedures that eliminate or reduce the risk of infection (2, 5-7).

Within epidemiological investigation, it is very important to identify the source of infection and transmission paths in a hospital environment. The basis of these investigations is to determine the relationship between the strains that have been isolated from patients with the epidemic outbreak. For this purpose, classical microbiological methods are based on the assessment of phenotypic characteristics of microorganisms, drug resistance mechanisms and molecular methods (5). Genotyping methods of strains are usually based on the restriction analysis of the entire genetic material of the microorganism or amplifying a genomic fragment using PCR (polymerase chain reaction) with suitable primers. The result of the analysis is to obtain patterns of DNA (fingerprints) of the tested strains indicating whether they are phylogenetically closely related and are derived from a single clone (5, 8, 9).

AIM

The aim of the study was to analyze the genetic relatedness of the strains of *Klebsiella pneumoniae* ESBL (+) isolated from patients hospitalized in the National Tuberculosis and Lung Diseases Research Institute (NTLDRI) at the Intensive Care Unit (ICU) and the Cardiology and Pneumology Intensive Care (OR).

MATERIAL AND METHODS

Research material consisted of 62 strains of *Klebsiella pneumoniae* ESBL (+) grown from 27 patients

hospitalized at the Intensive Care Unit (ICU) and the Cardiology and Pneumology Intensive Care (OR) for six months in 2013, when increased incidence of *Klebsiella pneumoniae* ESBL (+) in the materials of patients was observed in these wards (tab. 1). Identification of strains and antibiotic sensitivity were performed in an automated system Vitek 2 Compact. Molecular analysis was carried out using the RAPD method (8, 9).

Table 1. *Klebsiella pneumoniae* strains isolated in two Wards of Intensive Care for the period January-June 2013 with the ESBL (+) mechanism of resistance.

Ward	Number of primers (%)	Number of patients (%)	Material type/number	Phenotypes of isolated strains
OIT	47 (76)	21 (78)	Bronchoscopic material (29)	1. IPM, MEM, AN, ETP, SXT
			Wound swab (6)	2. IPM, MEM, AN, ETP
			Blood (7)	3. IPM, MEM, AN
			Urine (5)	
OR	15 (24)	6 (22)	Bronchoscopic material (6)	1. IPM, MEM, AN, ETP, SXT
			Wound swab (5)	2. IPM, MEM, AN, ETP
			Blood (1)	3. IPM, MEM, AN
			Urine (3)	
Total	62 (100)	27 (100)	62	–

IPM – imipenem, MEM – meropenem, ETP – ertapenem, AN – amikacin, SXT – co-trimoxazole

RESULTS

Basing on the epidemiological and microbiological analysis, 7 strains with the same mechanisms and patterns of resistance were selected for the molecular study, as isolated from 7 patients within 30 days in late April and early May. The strains were grown with various diagnostic materials, 4 with bronchoscopic material, 2 with wound swabs, 1 of blood. As they were different materials, possibility of transmission of strains between patients during medical procedures performed was excluded. All strains were resistant to beta-lactam antibiotics, including penicillins, cephalospo-

rins and monobactams. They only showed sensitivity to antibiotics of the carbapenem group and amikacin, an aminoglycoside (tab. 2).

The epidemiological investigation showed that these strains were grown in a short period of time, from neighboring patients at ICU and OR wards. The next stage of the investigation was to analyze the genetic relatedness of 7 *Klebsiella pneumoniae* ESBL (+) strains using the RAPD method (8, 9). The results of molecular studies are presented in figure 1.

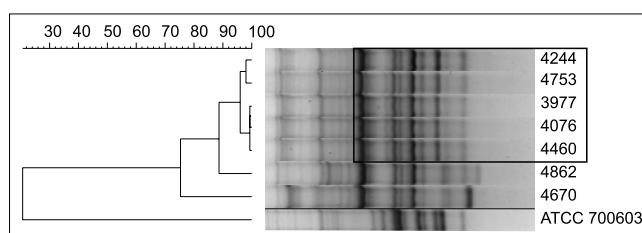


Fig. 1. Molecular patterns of *Klebsiella pneumoniae* ESBL (+) strains obtained in the RAPD method. The frame indicates five strains with identical DNA formulas. ATCC700603 – model *Klebsiella pneumoniae* strain.

Klebsiella pneumoniae ESBL (+) strains of 5 patients (4244, 4753, 3977, 4076, 4460) produced similar patterns of DNA. Other strains from two patients (4862, 4670) have individual molecular profiles.

DISCUSSION AND ANALYSIS

Nosocomial infections are one of the most serious problems of modern medicine. For many years, there has been a growing number of nosocomial infections, as evidenced by epidemiological data (7, 10). These infections consider mainly patients hospitalized at surgical wards and intensive care units. Due to the vast number of invasive procedures, including injections into the blood vessels, the establishment of urinary catheters, surgical wound dressing, patients treated in these units are more prone to infections caused by hospital strains of the than patients from conservative wards (2, 4, 6). The paper by Demir et al. shows that long stay in the hospital and mechanical ventilation were the main factors for the colonization of *Klebsiella pneumoniae* ESBL (+) strains (11). *Klebsiella pneumoniae* rods occupy a special place in the statistics

Table 2. *Klebsiella pneumoniae* strains isolated from patients within one month of 2013 with the ESBL (+) mechanism of resistance.

Date of strain isolation	Ward	No. of strain	Species of the isolated strain	Material type	Drug sensitivity – strain phenotype
28.04.2013	OIT	3977	<i>Klebsiella pneumoniae</i> ESBL (+)	Bronchoscopic material	IPM, MEM, ETP, AN
30.04.2014	OR	4076	<i>Klebsiella pneumoniae</i> ESBL (+)	Bronchoscopic material	IPM, MEM, ETP, AN
07.05.2013	OR	4244	<i>Klebsiella pneumoniae</i> ESBL (+)	Wound swab	IPM, MEM, ETP, AN
13.05.2013	OIT	4460	<i>Klebsiella pneumoniae</i> ESBL (+)	Bronchoscopic material	IPM, MEM, ETP, AN
21.05.2013	OIT	4753	<i>Klebsiella pneumoniae</i> ESBL (+)	Bronchoscopic material	IPM, MEM, ETP, AN
18.05.2013	OIT	4670	<i>Klebsiella pneumoniae</i> ESBL (+)	Wound swab	IPM, MEM, ETP, AN
26.05.2013	OIT	4862	<i>Klebsiella pneumoniae</i> ESBL (+)	Blood	IPM, MEM, ETP, AN

IPM – imipenem, MEM – meropenem, ETP – ertapenem, AN – amikacin

of hospital infections. Point Prevalence Surveys of the etiology of infections caused by *Enterobacteriaceae* from 2012 and 2013 are, respectively, 40.2 and 33.2%, including 30.0 and 24.4% of *Klebsiella pneumoniae*. Non-fermenting Gram-negative rods accounted for 15.2 and 18.8%, and Gram-positive cocci – 32.5 and 36.2% (www.antybiotyki.edu.pl). *Klebsiella pneumoniae* primarily causes a nosocomial urinary tract infection, pneumonia, infection of the skin and soft tissue infections, wound infections and sepsis (3, 4, 6, 9, 12). Most authors agree about the fact that pneumonia is the most common form of infection at intensive care units is, representing 30-49% (1, 2, 4, 6, 7). In 2014, at the Department of Microbiology, Institute of Tuberculosis and Lung Diseases, an epidemiology map was developed, covering the spectrum of bacterial patients treated in NTLDRI in the years from 2010 to 2013. This analysis showed a growing share of *Enterobacteriaceae* ESBL (+), including *Klebsiella pneumoniae* ESBL (+) in diseases of the respiratory system at the ICU and OR wards. Over three years, a more than two-fold increase in the incidence of *Enterobacteriaceae* ESBL (+) was found (fig. 2).

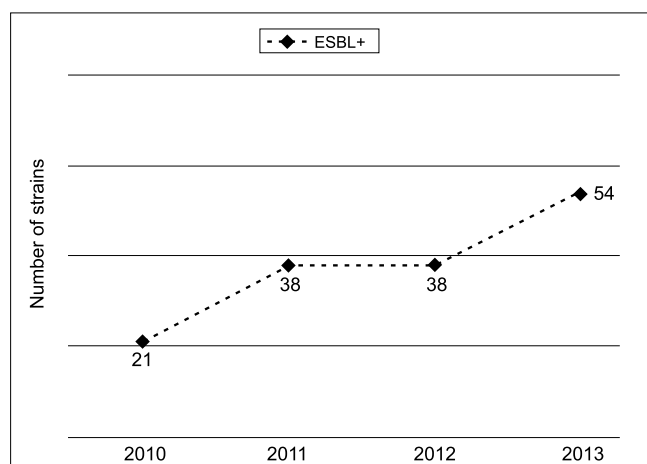


Fig. 2. The incidence of *Enterobacteriaceae* ESBL (+) isolated from bronchoscopic material and sputum material from patients at surgical wards of the NTLDRI in 2010-2013.

Nosocomial infections may affect individual patients or occur in the form of the so-called outbreaks, when the same pathogen is detected in at least two epidemiologically-linked cases. Such situations may include patients staying at the same time at a given ward and being in close contact (2, 5).

In the NTLDRI, during a routine microbiological diagnostics, increase in the incidence of *Klebsiella pneumoniae* ESBL (+) rods at ICU and OR wards was recorded. This observation influenced the decision to conduct molecular epidemiological investigations on *Klebsiella pneumoniae* ESBL (+) and to determine whether the infection of the population of the patients was caused by the same strain of *Klebsiella pneumoniae*. The molecular analysis carried out showed that the strains of *Klebsiella pneumoniae* from 5 patients had identical DNA patterns,

which indicates the transmission of strains between them. A more difficult task was to identify the source of the infection. It cannot be excluded that the hands of staff were the transmission vector in this case. What is more, the investigation revealed that the cleaning the room is a place common to the staff of the two wards, strains could be transferred there on the hands or clothing of personnel. The paper by Snitkin et al. describes a *Klebsiella pneumoniae* ESBL (+) nosocomial infection of 18 patients, of whom 11 died. The epidemiological investigation performed included a molecular analysis of strains isolated from material from 18 patients. The results showed that they had the same DNA profiles (13).

Pathogens responsible for nosocomial infections are transmitted mainly via the hands (14). Staff hand washing should be regarded as a prerequisite for personal hygiene. In all other situations, in the course of patient care from the medical staff, hygienic hand disinfection is required, as it is more effective than washing them (14-17). Disinfection should be applied before any action related to patient care and after. Hand hygiene is an effective way to prevent cross-transmission of microorganisms between staff and patients (15, 17). The paper by Skally et al. includes a retrospective epidemiological analysis which revealed two cases of transmission of *Klebsiella pneumoniae* ESBL (+) as a result of improper hand hygiene of hospital staff. The authors found that identification in patients being carriers of these strains is an important factor of surveillance over nosocomial infections (18). In practice, the hospital recommended hand hygiene procedures are often not respected or improperly used by staff (14-17). In the studies conducted, in samples taken from the environment, the presence of *Klebsiella pneumoniae* ESBL (+) was not detected in patients at ICU and OR wards. Despite this, we believe that *Klebsiella pneumoniae* ESBL (+) was present in the hospital environment and was the source of the outbreaks, as *Klebsiella pneumoniae* rods survive and multiply in all humid locations (sinks, humidifiers, drains).

Summaries of a number of epidemiological studies emphasize that *Klebsiella pneumoniae* ESBL (+) rods are often isolated from patients of intensive care units (2, 4, 6, 7). This phenomenon may be a consequence of the dynamic development that has been made in the diagnosis and treatment of serious illnesses, leading to an increase in the number and prolonged lives of people with weakened immune systems (6, 7). Due to severity of infections caused by *Klebsiella pneumoniae* ESBL (+) during hospitalization, which can be life-threatening, especially in sepsis, meningitis and pneumonia, bacteriological examinations are crucial (4-7, 19). In the light of the build-up of microbial resistance to antibiotics, stable and professional control of hospital ecosystems, environmental testing and registration of infections, especially infections caused by alarm pathogens, are required (5).

Among the pathogens responsible for nosocomial infections at intensive care units non-fermentative

Acinetobacter spp. should also be taken into account. According to numerous reports, they are often isolated at ICUs. These bacteria have a small life footprint and the ability to colonize patients and medical equipment (20, 21).

Supervision over nosocomial infections should be based on a hospital infection control program and should be coordinated by a special team on nosocomial infections. The main objective of this program is to monitor the prevalence of infections at hospital wards, and particularly rapid provision of information to doctors about the emergence of a new strain of a pathogen or high drug resistance.

Results of epidemiological surveillance allow for epidemic risk assessment in the hospital and the im-

plementation of preventive procedures. They are also the basis for the development of a hospital formulary, perioperative prophylaxis and empiric therapy principles at the wards (5, 7).

CONCLUSIONS

1. Among the strains of *Klebsiella pneumoniae* ESBL (+), isolated from 27 patients at the ICU and OR wards, the same molecular patterns were found in 5, indicating transmission of infection.
2. Molecular analysis and analysis of resistance patterns of strains should be obligatory in epidemiological investigations and in the supervision of nosocomial infections.

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