Antimicrobial susceptibility and genetic similarity of ESBL-positive *Klebsiella pneumoniae* strains

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Abstract

Introduction: Twenty-eight isolates of extended-spectrum β-lactamase (ESBL)-positive *Klebsiella pneumoniae* were studied.
Material and methods: The strains were cultured from different clinical specimens obtained from children hospitalised at the University Hospital in Bydgoszcz. Seventeen strains were isolated from colonization and eleven from clinical infection. Isolation and identification of bacteria were performed using routine methods at the clinical microbiology laboratory. Production of ESBL was assessed using the double disk synergy test. The susceptibility to imipenem and tigecycline was tested by the Etest. The susceptibility to gentamicin and ciprofloxacin was tested by the agar dilution method. The genomic DNA was extracted from the strains separated by pulsed-field gel electrophoresis (PFGE) after digesting with *XbaI* endonuclease.
Results: Among analysed *K. pneumoniae* strains all were susceptible to imipenem, 21 (75.0%) were susceptible to tigecycline, 14 (50.0%) to gentamicin and 5 (17.9%) to ciprofloxacin. Molecular typing results revealed a great genetic diversity among *K. pneumoniae* isolates. All repeated PFGE patterns were detected in seven *K. pneumoniae* isolates. Among identical *K. pneumoniae* strains four susceptibility patterns were detected.
Conclusions: The results of the study suggest that establishing strains' similarity in epidemiological investigations should be based on results obtained by several methods, and that each phenotyping method should be complemented with genetic research.

Key words: *Klebsiella pneumoniae*, ESBL phenotype, susceptibility to antibiotics, pulsed-field gel electrophoresis.

Introduction

Klebsiella pneumoniae rods are one of the most important bacteria which cause nosocomial infections, especially at paediatric units. β-Lactamases are the most frequent source of resistance to β-lactam antibiotics. Different β-lactamases are known: penicillinases, extended-spectrum β-lactamases (ESBL), cephalosporinases (AmpC), metallo-β-lactamases (MBL) and carbapenemases (KPC). Each of them can be produced by *Klebsiella* strains. *Klebsiella pneumoniae* are one of the most frequent ESBL producers, including in Poland [1-3]. Strains with this phenotype hydrolyse penicillins and some cephalosporins, and are normally not inhibited by β-lactamase inhibitors. ESBL-positive strains are often resistant to non-β-lactam antibiotics, so treatment of these infections appears to be very

difficult because of the strains' multidrug resistance. Since ESBL-producing strains were first recognized, their emergence and rapid dissemination have been responsible for numerous outbreaks of infection throughout the world [4-6]. Strains with this phenotype generally remain susceptible to only a few antimicrobial agents, some of which are not always recommended for use in children.

Phenotypic methods such as biotyping and serotyping have been providing the mainstay in descriptive epidemiology of bacteria. Genetic methods allow one to evaluate the relatedness of strains and the epidemiological situation in a hospital. Pulsed-field gel electrophoresis (PFGE) is a genetic typing method that is widely used as a molecular epidemiological tool for studying the genetic diversity of *K. pneumoniae* [7, 8].

The purpose of this dissertation was to evaluate antimicrobial susceptibility and genetic similarity of ESBL-positive *K. pneumoniae* strains isolated from colonization and infections from children hospitalised at the Paediatric Surgery Unit at the Dr Jurasz University Hospital in Bydgoszcz in a period of 15 months.

Material and methods

The study included 28 *K. pneumoniae* strains isolated from 18 children. Isolation and identification of bacteria were performed using routine methods at the clinical microbiology laboratory. The isolates were identified with commercial ID32E tests (bioMérieux). The antimicrobial susceptibility tests for gentamicin and ciprofloxacin were performed by the agar dilution method. Minimal inhibitory concentration (MIC) for imipenem and tigecycline was determined by the Etest (AB Biodisk). Susceptibility breakpoints for antibiotics were established according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing [9]. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as reference strains. For every strain the ESBL producing ability was determined by means of two disks and the EUCAST method.

Genetic relatedness was assessed by PFGE using *XbaI* enzyme. Electrophoresis conditions were as follows: 14°C at 6 V/cm for 20 h with pulse time ranging from 2 s to 35 s. As a reference chromosomal DNA pattern Lambda Ladder standard (Bio-Rad) was used. Results were interpreted according to Tenover *et al.* [10] and Molecular Analyst Fingerprinting (MAF, Bio-Rad).

Results

Seventeen *K. pneumoniae* (60.7%) strains were isolated from colonization. These strains were cultured from: throat swabs – 9 (32.2%), rectal swabs – 6 (21.4%) and stool samples – 2 (7.1%). Eleven (39.3%) strains were isolated from children with clinical symptoms of infection from: blood – 6 (21.4%), urine – 3 (10.8%) and cerebrospinal fluid samples – 2 (7.1%). All the *K. pneumoniae* strains in this study demonstrated ESBL-mediated resistance and most of them were resistant to ciprofloxacin.

Among the analysed strains all of them were susceptible to imipenem, 21 (75.0%) to tigecycline, 14 (50.0%) to gentamicin and 5 (17.9%) to ciprofloxacin. Four *K. pneumoniae* strains were intermediate to tigecycline. The results of antimicrobial susceptibility testing are presented in Table I. Imipenem MICs were 0.19-1 mg/l. Tigecycline MICs varied from 0.25 mg/l to 8 mg/l. Gentamicin and ciprofloxacin MICs were 0.125-256 mg/l.

Against the analysed *K. pneumoniae* strains, imipenem revealed the highest activity, with MIC_{50S} 0.19 mg/l and MIC_{90S} 0.38 mg/l. Against the analysed *K. pneumoniae* strains, ciprofloxacin showed the lowest activity, with MIC_{50S} 2 mg/l and MIC_{90S} 16 mg/l.

According to the PFGE testing results, the study strains were divided into four subgroups: 7 (25.0%) identical strains (group A – lines 9, 10, 10*, 12, 12*, 13, 13*); 9 (32.2%) closely related strains (group B – lines 2, 8, 8*, 9*, 11, 11*, 14*, 16, 16*); 4 (14.2%) possibly related strains (group C – lines 1, 5, 7, 7*); and

Table I. Antimicrobial susceptibility of *K. pneumoniae* ESBL-positive strains (n = 28)

Antibiotics				Susceptibility pattern	Number of strains
Imipenem	Gentamicin	Tigecycline	Ciprofloxacin		
S	S	S	S	a	2
S	R	S	S	b	2
S	S	R	S	c	1
S	S	S	R	d	9
S	S	R	R	e	2
S	R	I	R	f	4
S	R	S	R	g	8

S – susceptible, I – intermediate, R – resistant

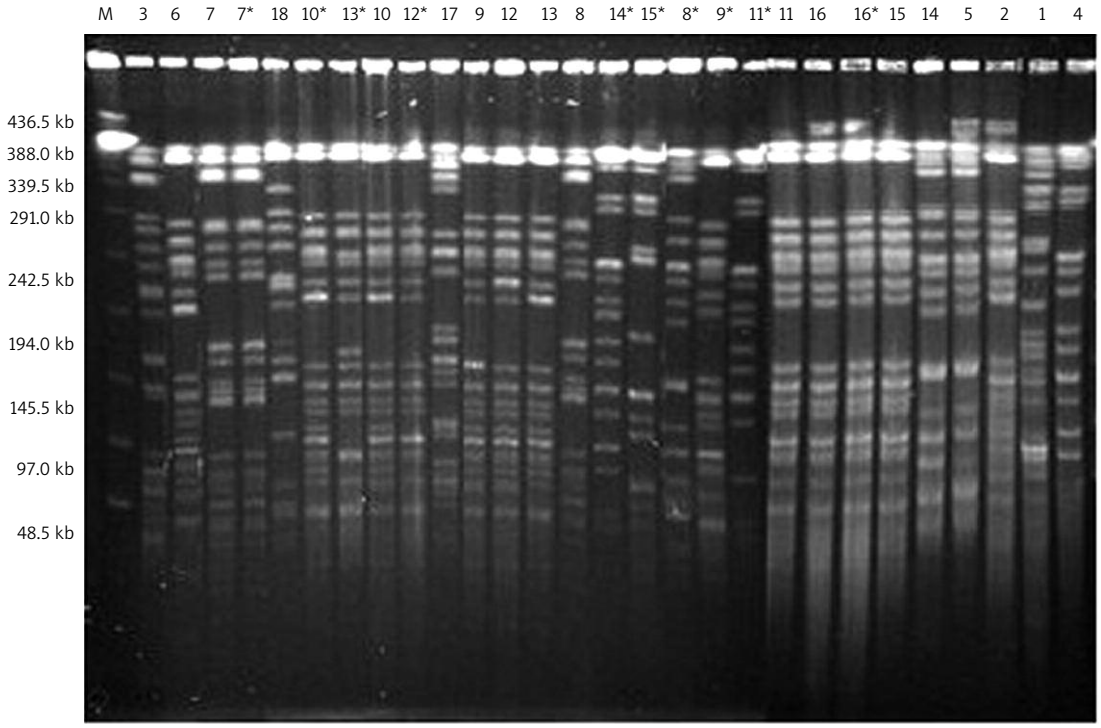

Figure 1. PFGE fingerprinting of *XbaI* –digested DNA from *K. pneumoniae* ESBL-positive strains (*n* = 28)
*M – Lamba Ladder stander, 1-16 strain number, strains marked *were isolated from infections*

8 (28.6%) unrelated strains (group D – lines 3, 4, 6, 14, 15, 15*, 17, 18). The results of PFGE and MAF are presented in Figures 1 and 2. Among *K. pneumoniae* strains, which were isolated from colonization, 3 identical, 5 closely related, 2 possibly related and 7 different strains were detected according to Tenover *et al.* [10]. Among *K. pneumoniae* strains which were cultured from infections, 4 identical, 4 closely related, 2 possibly related and 1 different strains were detected according to Tenover [10]. Six strains showed 95% similarity according to MAF.

Among identical *K. pneumoniae* strains four susceptibility patterns were noted (b, d, e, g). All identical strains were susceptible to imipenem. Among closely related strains four susceptibility patterns (d, e, f, g) were detected. These strains were susceptible to imipenem and resistant to ciprofloxacin. Among possibly related strains two susceptibility patterns were detected (a, g). These strains were susceptible to imipenem and tigecycline. Among different *K. pneumoniae* strains three susceptibility patterns were detected (c, d, f). All of these strains were susceptible to imipenem.

The *K. pneumoniae* strains that were isolated from the same child from colonization and infection had the same susceptibility pattern (Table II). Among the analysed strains "d" and "g" susceptibility patterns dominated. These patterns were detected in 9 (32.1%) and 8 (28.6%) strains, respectively. The *K. pneumoniae* strains cultured from the same child from colonization and infection belonged to the same genetic group in 8 cases.

Discussion

Klebsiella pneumoniae bacilli can be typed by means of various methods. The commonly applied typing methods rely on biochemical properties,

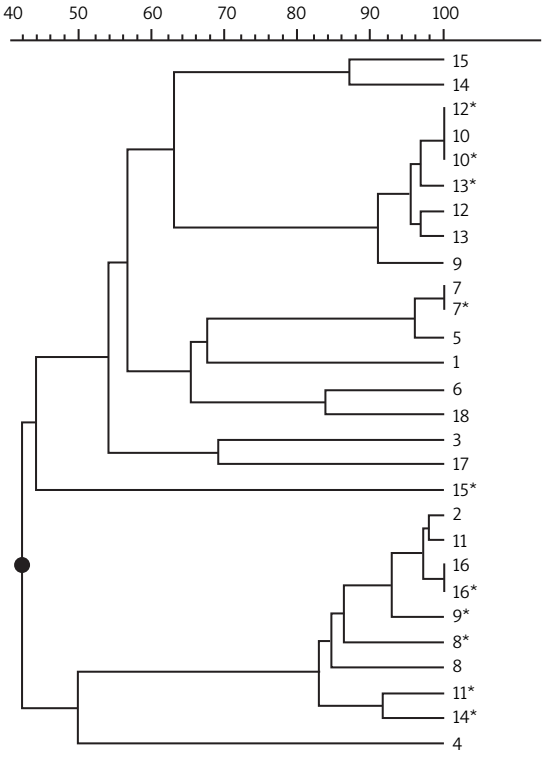

Figure 2. Dendrogram of the *K. pneumoniae* strains

Table II. Antimicrobial and genetic patterns of *K. pneumoniae* ESBL-positive strains (*n* = 15)

Strain number	Genetic pattern	Susceptibility pattern	Data of isolation	MIC value [mg/l]			
				Imipenem	Gentamicin	Tigecycline	Ciprofloxacin
1	D	c	X 2006	0.25	0.125	4	0.125
2	B	f	XII 2006	0.75	8	1.5	64
3	D	f	X 2006	0.125	8	2	64
7	C	a	IX 2006	0.19	0.25	0.047	0.125
7*	C	a	X 2006	0.19	0.5	0.047	0.5
8	D	d	IX 2006	0.19	0.25	1	2
8*	D	d	IX 2006	1	1	1	2
9	A	e	IX 2006	0.75	2	8	4
9*	B	e	IX 2006	0.75	2	8	4
10	A	b	X 2006	0.19	32	1	0.5
10*	A	b	X 2006	0.19	64	1	0.5
11	B	d	IX 2006	0.125	0.5	0.5	16
11*	B	d	X 2006	0.125	1	0.5	64
17	D	d	X 2006	0.19	2	0.5	8
18	A	d	IX 2006	0.19	2	1	16

*Strains marked with the same numbers came from one child. Strains marked * were isolated from infections*

Table III. Antimicrobial and genetic patterns of *K. pneumoniae* ESBL-positive strains (*n* = 13)

Strain number	Genetic pattern	Susceptibility pattern	Data of isolation	MIC value [mg/l]			
				Imipenem	Gentamicin	Tigecycline	Ciprofloxacin
4	D	d	X 2007	0.75	2	1	32
5	D	d	X 2007	0.38	0.125	0.25	4
6	A	d	X 2007	0.38	0.125	0.5	4
12	A	g	IX 2007	0.25	16	0.75	2
12*	B	g	X 2007	0.25	32	0.75	2
13	A	g	X 2007	0.19	256	1	4
13*	A	g	X 2007	0.19	256	1	8
14	C	g	XI 2007	1	32	0.5	32
14*	C	g	XI 2007	1	256	1	256
15	D	f	XI 2007	0.19	8	1.5	32
15*	D	f	XI 2007	0.19	16	2	32
16	B	g	XI 2007	0.125	8	1	2
16*	B	g	XI 2007	0.125	16	1	4

*Strains marked with the same numbers came from one child. Strains marked * were isolated from infections*

phage typing, and serological typing or drug sensitivity profiles. These methods, however, do not always provide a proper interpretation of an epidemiological situation. Molecular biology methods, including PFGE, allow for genetic differentiation of strains belonging to the same species. They are not commonly used in laboratories due to the lack of equipment and insufficient staff training. The studies of Demirdag and Hosoglu [2], Dzierżanowska *et al.* [3] and Ktari *et al.* [11] prove the usefulness of both methods applied in this study for *K. pneumoniae* strain typing. Seven drug sensitivity profiles were determined among 28 *K. pneumoniae* strains in this study. On this basis it can be assumed that certain *K. pneumoniae* strains survive in the clinic environment in which colonised patients or patients infected with these bacilli stayed. Strain typing on the basis of the drug sensitivity profile was one of the most frequently used methods in hospital strains. The introduction

of genetic methods provided new opportunities within their typing and similarity determination. The results of PFGE chromosomal DNA isolation obtained in the study prove that during 15 months genetically identical *K. pneumoniae* strains were isolated from different children, in the case of both colonisation and infection. This may prove maintenance of colonization and dissemination of strains in children treated in the clinic involved in the study. From the results it was deduced that strains classified in the same group on the grounds of chromosomal DNA patterns can have different drug sensitivity profiles. Similar results were obtained by Bagattini *et al.* [12] when studying *K. pneumoniae* ESBL-positive strains isolated from children treated in the neonatal intensive care unit. No direct correlation was found between PFGE profiles and antibiotic susceptibility patterns. Isolates with identical antibiotypes belonged to different PFGE types. The various drug sensitivity profiles obtained for genetically identical *K. pneumoniae* strains may result from development of plasmid-encoded resistance genes. Non-compliance of a sanitary regime in the department as well as patients' migration within departments may affect development of drug sensitivity resistance genes through clinic strains. Equally significant is the ability of strains with different resistance mechanisms to survive in the hospital environment. Differences in MIC values for antibiotics of *K. pneumoniae* strains isolated from the same patient may suggest that survival of strains in a human body facilitates development of drug resistance during antibiotic therapy.

In conclusion, the results of the study suggest that establishing strains' similarity in epidemiological investigations should be based on results obtained by several methods, and that each phenotyping method should be complemented with genetic research.

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