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- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

## Diversity of extended-spectrum beta-lactamase-producing *Escherichia coli* rods\*

### Zróźnicowanie szczepów *Escherichia coli* wytwarzających beta-laktamazy o rozszerzonym spektrum substratowym

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#### Summary

**Introduction:**

The aim of the study was to evaluate genetic relatedness and antimicrobial susceptibility of extended-spectrum beta-lactamase-producing *E. coli* strains isolated from patients hospitalized in the University Hospital in Bydgoszcz (Poland).

**Material and methods:**

The study included 33 extended-spectrum beta-lactamase-producing *E. coli* strains isolated from 31 patients. The chromosomal DNA was extracted from the strains and separated by pulsed-field gel electrophoresis. Antimicrobial susceptibility testing was performed by the agar dilution method and carried out according to the European Committee on Antimicrobial Susceptibility Testing recommendations.

**Results:**

According to the pulsed-field gel electrophoresis results, 32 distinct pulsotypes were revealed. Based on Molecular Analyst Fingerprinting software analysis, the studied isolates were divided into four subgroups: 6 (18.2%) isolates showing similarity greater than 90% (group A); 19 (57.6%) showing 80-90% similarity (group B); 7 (21.2%) showing 70-79% similarity (group C); and one isolate with less than 70% similarity (group D). Among *E. coli* isolates showing similarity greater than 90%, four antimicrobial patterns were noted. Among the isolates showing 80-90% similarity, 18 antimicrobial patterns were observed. *E. coli* isolates showing 70-79% similarity presented 6 antimicrobial patterns.

**Conclusions:**

Our results show a high degree of genetic diversity of extended-spectrum beta-lactamase-producing *E. coli* isolates. However, based on a similarity of  $\geq 80\%$ , almost 75% of *E. coli* isolates were clonally related. Although it is difficult to identify definitive transmission events based on the recovery of indistinguishable pulsed-field gel electrophoresis types alone, we speculate that extended-spectrum beta-lactamase-producing *E. coli* strains may have disseminated throughout the hospital.

**Key words:**

*escherichia coli* • extended spectrum beta-lactamases • genetic relatedness • pulsed-field gel electrophoresis • antimicrobial susceptibility

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## INTRODUCTION

*Escherichia coli* is a Gram-negative rod, the dominant component of the physiological microflora of humans' and animals' digestive tract. In addition to the commensal *E. coli* strains, there are pathogenic strains within a species, responsible for diarrhea, enteritis (intestinal pathogenic *E. coli*, IPEC) and extra-intestinal infections such as wound infections, urinary tract infections, neonatal meningitis, bacteremia and sepsis (extra-intestinal pathogenic *E. coli*, ExPEC) [4].

The most important issue, from the clinical and epidemiological point of view, is the resistance of *E. coli* to beta-lactams. It is most often associated with beta-lactamases synthesis, including extended-spectrum beta-lactamases (ESBLs). Strains that produce ESBLs are widely disseminated, especially in a hospital environment, where they may even cause an endemic. They are responsible for therapeutic failure, especially with the use of beta-lactam antibiotics, and are often associated with increased morbidity, mortality and health care-associated costs [10,11].

Phenotypic and genetic analysis of the strains provides information on their virulence, antibiotic resistance, origin and clonal similarity. Typing of the strains is particularly indispensable in an epidemiological investigation, as it allows determination of the focus of the infection and the way it spreads. Genetic methods enable evaluation of the relatedness of the strains and the epidemiological investigation. Pulsed-field gel electrophoresis (PFGE) for example has been considered as the 'gold standard' among molecular typing methods for a variety of clinically important bacteria, including *E. coli* [9].

The aim of the study was to evaluate genetic relatedness and antimicrobial susceptibility of ESBL-positive *E. coli* strains isolated from patients hospitalized in Dr. A. Jurasz University Hospital No. 1 in Bydgoszcz (Poland).

## MATERIAL AND METHODS

The study included 33 ESBL-positive *E. coli* strains isolated from 31 patients at the Department of Clinical

Microbiology of Dr. A. Jurasz University Hospital No. 1 in Bydgoszcz (Poland) between 2011 and 2013 (January 2011 to January 2013). The origin of the *E. coli* strains (clinical samples and clinical units) is shown in Fig. 1.

Isolation of the bacteria was performed using routine methods at the clinical microbiology laboratory. The isolates were identified with the VITEK2 GN colorimetric cards that were read out with the VITEK2 Compact automated system (bioMérieux). Antimicrobial susceptibility testing was performed by the agar dilution method and carried out according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (version 3.1 2013) [7]. For every strain, the ESBL synthesis was determined by means of a double-disk synergy test (with amoxicillin/clavulanic acid, cefotaxime, ceftazidime and cefepime) and the EUCAST method. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the reference strains.

The molecular typing was performed by PFGE. Analysis of genomic profiles was adapted from the previously published procedure [6]. The isolated chromosomal DNA was digested for 20 h with *Xba*I enzyme in Tango Buffer (Fermentas) at a final concentration of 50 U/ml. The electrophoresis conditions were as follows: pulse time ranging from 2 s to 35 s, run time 20 h, gradient 6 V/cm, temperature 14°C. As a reference chromosomal DNA pattern, the Lambda Ladder standard (Bio-Rad) was used. PFGE was performed with the CHEF Mapper (BioRad). The results were analyzed using the Molecular Analyst Fingerprinting (MAF) software (Dice coefficient, position tolerance 1.5%) (Bio-Rad). Strains indicating 100% genetic profile similarity were considered identical.

## RESULTS

Among the analyzed *E. coli* strains, 29 different antimicrobial patterns were noted. All the analyzed strains were susceptible to imipenem and meropenem, 29 (87.9%) strains were susceptible to tigecycline, 28 (84.8%) to nitrofurantoin, 23 (69.7%) to ertapenem, 21 (63.6%) to piperacillin with tazobactam and netilmicin, 19 (57.6%) to gentamicin, and 17 (51.5%) to ami-

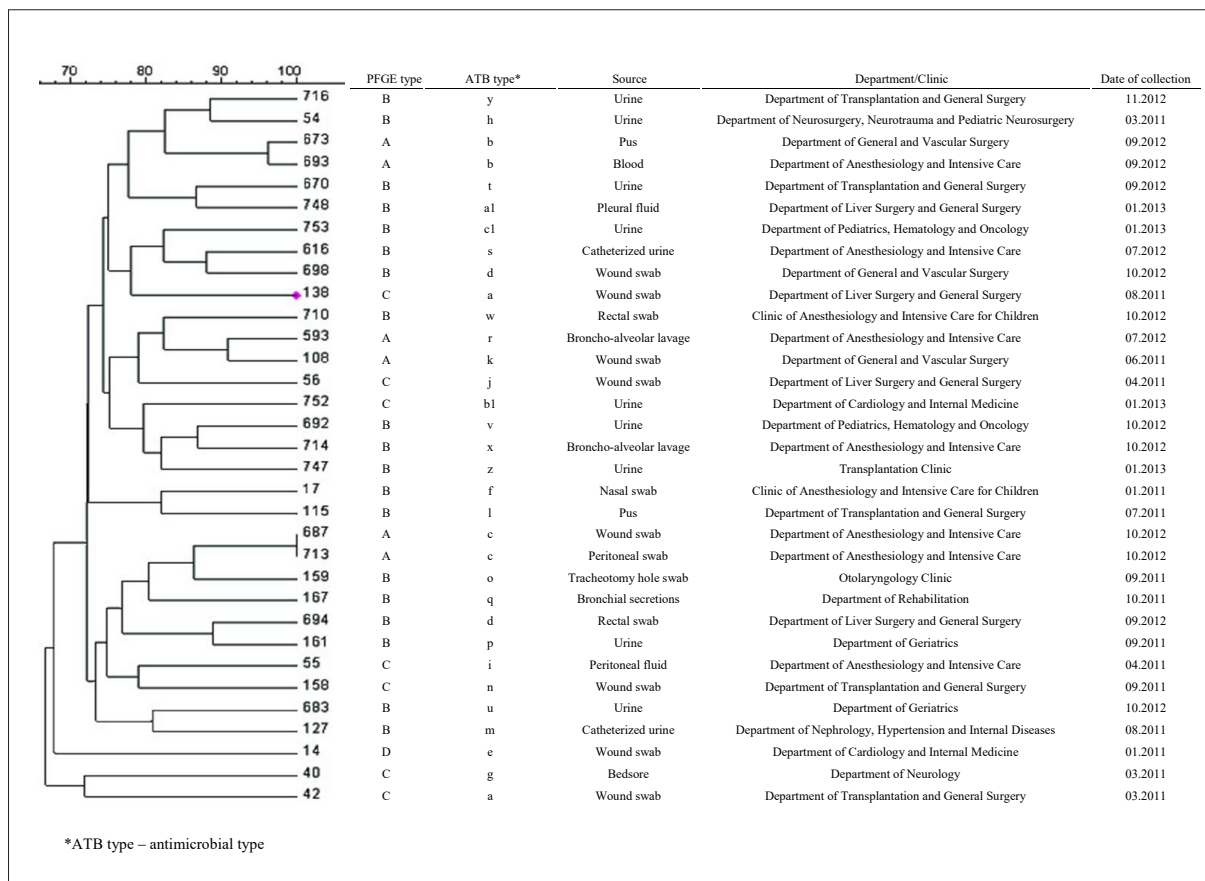


Fig. 1. Genetic relatedness, antimicrobial patterns and the origin of ESBL-positive *E. coli* isolates (n=33)

kacin and tobramycin. Thirty-one (93.9%) strains were non-susceptible to cefotaxime, 29 (87.9%) to amoxicillin with clavulanic acid and ticarcillin with clavulanic acid, 23 (69.7%) to trimethoprim/sulfamethoxazole and ceftazidime, 18 (54.5%) to cefepime and 17 (51.5%) to ciprofloxacin. All the *E. coli* strains showed resistance to ampicillin, piperacillin, ticarcillin and cefuroxime. The results of antimicrobial susceptibility testing are presented in Table 1.

The results of PFGE and MAF analysis are presented in Fig. 2. According to the PFGE results, 32 distinct pulsotypes were revealed. Based on MAF analysis, the studied isolates were divided into four subgroups: 6 (18.2%) isolates showing similarity greater than 90% (group A – isolates: 108, 593, 673, 687, 693, 713); 19 (57.6%) showing 80-90% similarity (group B – isolates: 17, 54, 115, 127, 159, 161, 167, 616, 670, 683, 692, 694, 698, 710, 714, 716, 747, 748, 753); 7 (21.2%) showing 70-79% similarity (group C – isolates: 40, 42, 55, 56, 138, 158, 752) and one with less than 70% similarity (group D – isolate: 14). The isolates collected from cases of colonization (17, 694, 710) belonged to group B. The results of typing *E. coli* strains by phenotypic and genetic methods are shown in Table 1, Fig. 1 and 2. Among *E. coli* isolates showing similarity greater than 90%, four antimicrobial patterns were noted. Among the analyzed isolates showing 80-90%

similarity, 18 antimicrobial patterns were detected. *E. coli* isolates showing 70-79% similarity presented 6 antimicrobial patterns.

## DISCUSSION

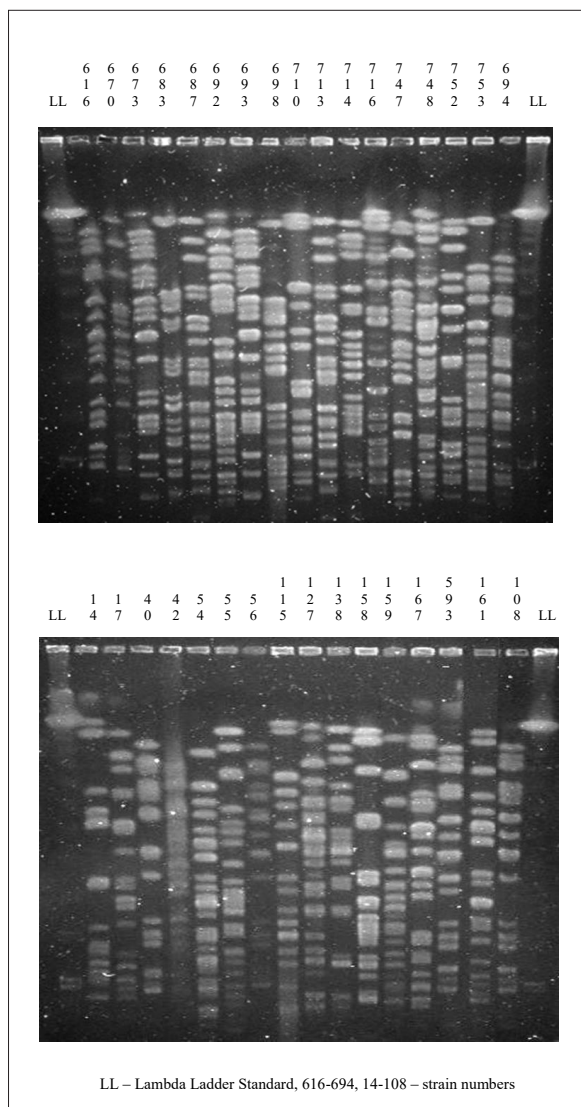
The prevalence of ESBLs-positive strains among clinical isolates, including *E. coli*, has increased significantly [5]. ESBL-producing *E. coli* strains are an increasing cause of healthcare-associated infection, where they may even cause an endemic. Therefore, in the present study, ESBL-positive *E. coli* strains were subjected to a phenotypic (analysis of susceptibility patterns) as well as a genotypic typing method (PFGE). Comparison of the genetic profiles with the results of the drug resistance evaluation of the analyzed strains showed the variability of antimicrobial profiles among isolates with high genetic similarity. It may be explained by the differences in the composition and structure of extra-chromosomal mobile genetic elements, such as plasmids and integrons, containing for example resistance genes. Since genetic material of *E. coli* is constantly rearranged, for example through mutation (e.g. deletions, insertions), specific genetic profiles are a variable value in a given environment (e.g. a hospital).

In studies by Burke et al. [2] and Peirano et al. [8], closely related ESBL-positive *E. coli* isolates had different anti-

**Table 1.** Antimicrobial profiles of ESBL-positive *E. coli* strains (n=33)

	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	a1	b1	c1	
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Amoxicillin/ clavulanic acid	R	R	R	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	
Piperacillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Piperacillin/ tazobactam	R	I	I	S	S	S	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
Ticarcillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Ticarcillin/ clavulanic acid	R	R	R	R	R	S	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	
Cefuroxime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Cefotaxime	R	R	R	R	R	R	I	R	R	R	R	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Ceftazidime	R	R	R	S	R	R	S	R	S	S	I	S	S	R	R	R	R	S	R	R	R	I	R	R	I	S	R	R	S	
Cefepime	S	I	I	I	S	S	S	S	R	R	S	R	S	S	S	S	S	S	I	S	I	R	R	I	I	S	R	R	S	
Imipenem	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
Meropenem	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
Ertapenem	R	S	S	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	I	S	
Gentamicin	S	R	R	S	S	I	S	I	R	S	S	S	S	S	S	S	R	S	S	R	R	R	R	S	R	S	S	R	S	
Amikacin	S	I	I	S	S	I	I	I	S	S	I	S	S	S	S	I	S	I	I	S	I	I	I	I	S	I	S	S	I	
Netilmicin	S	R	S	S	S	R	I	R	S	S	R	S	S	S	S	R	S	S	I	S	R	S	R	S	R	S	S	R	S	
Tobramycin	S	R	R	S	S	R	R	R	S	S	R	S	S	S	R	S	R	S	R	R	S	R	R	S	R	S	S	R	S	
Ciprofloxacin	S	R	R	S	S	R	R	S	R	R	R	S	S	R	S	R	S	R	R	R	R	R	S	R	S	S	S	S	I	S
Trimethoprim/ sulfamethoxazole	R	S	R	S	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R	S	R	S	R	R	S	R	S
Tigecycline	S	S	S	S	S	I	R	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S
Nitrofurantoin	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S	R	S	S	S	R	R	S	S	S	S	S	S	S	S	S
Strain number	42, 138	673, 693	687, 713	694, 698	714	716	747	748	752	753																				

S – susceptible, I – intermediate, R – resistant



**Fig. 2.** PFGE fingerprinting of *XbaI* – digested DNA from ESBL-positive *E. coli* isolates (n= 33)

microbial profiles. In our study, 32 unique PFGE patterns were noted among 33 *E. coli* isolates. ESBL-positive *E. coli* isolates were collected in the time range of 24 months. It shows a high degree of genetic diversity of the isolates studied in our work. However, based on a similarity of  $\geq 80\%$ , almost 75% of *E. coli* isolates were clonally related.

Brolund et al. [1] in their study obtained a smaller degree of heterogeneity of the ESBL-positive strains.

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However, the strains for their study were collected only over a period of three months and the degree of similarity in the majority of the analyzed isolates was greater than 85%. Among the 28 ESBL-positive *E. coli* strains collected in six months, Carattoli et al. [3] identified only 11 different PFGE profiles (the degree of similarity between all the strains was in the range of 85-100%). In the studies by Peirano et al. [8] more than 69.2% of ESBL-positive isolates collected in 2008 (12 months) showed similarity greater than 80%. Similar results were obtained in the study conducted by Burke et al. [2]; the similarity of 64.0% of the isolates collected within two years exceeded 80%.

In our study clinical specimens were delivered from different departments and outpatients. The majority of the isolates (36.4%) were collected from the patients with urinary tract infection, mainly (7 isolates, 21.2%) from the Department of Anesthesiology and Intensive Care (ICU). However, analysis of the isolates with 80-90% similarity showed a different origin. Most of the clinical specimens were derived from the patients of Intensive Care and surgical clinics.

By comparing the genetic profiles of *E. coli* isolates in terms of their place of origin, the way of their spread in the environment can be presumed. In this study, three pairs of ESBL-positive *E. coli* isolates of genetic similarity greater than 90% were identified. Paired isolates exhibiting 100% (687, 713) and 96% (673, 693) similarity were collected from different clinical specimens of the same patients. The first pair was derived from an ICU patient, in the second pair one of the isolates was obtained from the ICU and the other from the Department of General and Vascular Surgery (GVSU) (the patient was transferred from the ICU to the GVSU). Among the pairs of isolates with 91% similarity the first one was obtained from an ICU patient while another was derived from a GVSU patient.

Although it is difficult to identify definitive transmission events based on the recovery of indistinguishable PFGE types alone, we speculate that ESBL-positive *E. coli* strains may have disseminated throughout the hospital. The comparison of the origin of certain pairs of strains indicates the possibility of bacteria transmission from the ICU to the surgical clinics. These observations confirm that the branches of the greatest risk of infection include intensive care, so one should constantly monitor emerging infections among patients treated in the ICU.

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The authors have no potential conflicts of interest to declare.