Genetic similarities of *Escherichia coli* isolated from hospitalized patients

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ABSTRACT

Introduction: *Escherichia coli* is a component of human physiological flora. Pathogenic *E. coli* strains are a significant etiologic factor for numerous infections, mainly the urinary system, digestive system, respiratory system as well as bacteraemia and post-operative infections.
Purpose: To compare the genetic similarity of *Escherichia coli* strains, isolated from biological material collected for routine microbiological diagnostics.
Materials and methods: The examination performed on the *Escherichia coli* strains, isolated from material collected from patients hospitalized in various clinics and delivered for routine laboratory diagnostics. The analysis was conducted using the ADRSSR method.
Results: As a result of the analysis of genetic similarities of examined strains using the ADRSSR method, nine clones were distinguished, clones A and B considered being most numerous. Clone A was predominant in samples from internal diseases clinics while cloning B – from neonatological clinics.
Conclusions: The results point to a significant role of monitoring of homogeneity of bacteria strains isolated in the range of the health care providers. It is directly connected with the safety of hospitalized patients as well as effectiveness and course of the treatment. The use of the ADSRRS method gives the opportunity of early detection of the moment of colonization in the monitored place.
Keywords: *Escherichia coli*, ADSRRSA

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INTRODUCTION

Escherichia coli, originally called *Bacterium coli commune,* was discovered by Theodor Escherich in 1885 [1]. In 1930, during the International Congress of Microbiology in Paris, the name was changed into *Escherichia coli* [2].

Escherichia coli is a Gram-negative non-sporulating rod-shaped bacterium of 20-minut generation time, belonging to *Enterobacteriaceae* family. It is an aerobic or facultatively anaerobic bacterium with the ability to move and grow at the temperature of 7 to 50^0C. Approximately 95% of strains can disintegrate lactose [3].

The division into serotypes was introduced due to differences in in the structure of somatic O-antigens, capsular K-antigens, and flagellar H-antigens. However, not every type of antigen (K and H) is present in all the strains. Nowadays, over 700 *E. coli* serotypes are classified [4].

Escherichia coli is the environmental bacterium that constitutes a component of human physiological flora, however numerous pathogenic *E. coli* strains are responsible for various infections, including systemic ones. In-house infections of hospitalized patients are a significant and growing clinical problem that hinders patients recovery and spoils the treatment process. A longer stay in hospital and necessity of further therapy and medical procedures incorporation give additional social and economic costs.

Pathogenic *Escherichia coli* may be responsible for infections of the urinary system, digestive system, respiratory system, the brain and brain membranes, bacteraemia, and post-operative infections [5].

It is possible thanks to different factors of bacterial virulence, like invasive and adhesion molecules, toxins, anti-phagocytic and anti-serous factors, anti-immunological and genetic factors, mobility, and chemotaxis [6].

The following pathotypes can be distinguished considering induced infections: EPEC, ETEC, EIEC, EHEC, EAEC, UPEC, AIEC, STEC, NMEC [7,8–10].

The aim of the study was the comparison of genetic similarities of *Escherichia coli* strains, isolated from a clnical material that underwent routine microbiological diagnostics using the ADSRRS method. The ADSRRS method (Amplification of DNA Surrounding Rare Restriction Sites) is a modern molecular analytic method developed by Płucienniczak and Masny, based on supression of amplification of parts of fragments in PCR reaction [11].

This is the first study concerning hospital strains of E.coli using the ADSRRS method.

MATERIALS AND METHODS

In the study, we analysed 50 strains of Escherichia coli isolated from material collected from patients hospitalized in clinics and departments of the teaching hospital. All the strains used in the study underwent species identification by using the automatic microbiological system Vitek and GNI card designed for identification of Gram-negative microorganisms.

Total bacterial DNA used in the study was isolated using a method based on the ability to bind nucleid acids by silica. The process was carried out in accordance with the methodics described by Boom [12] in the modification by Masny and Płucienniczak [13].

The quality of isolated DNA was checked using electrophoresis in agarose gel [14] while its concentration and purity in obtained solutions were determined as the ratio of A260/A280 values[15].

The ADSRRS method (Amplification of DNA Fragments surrounding rare restriction sites) The method was developed by Alexander Masny and Andrzej Płucienniczak as a future alternative for pulsed-field gel electrophoresis (PFGE) [11].

It iimits the number of replicated fragments in PCR reaction to the number that enables univocal analysis of obtained products with the use of electrophoresis in polyacrylamide or agarose gel stained with ethidium bromide. The basic elements of this method are restrictive endonuclease, adaptors, and primers of PCR reaction.

The essential element of the method is supression which takes place in case of ligation of long complementary sequences on both ends of the product. It results from markedly higher affinity to re-association within one molecule than between different molecules. Thus, a structure of so called „tennis racket" appears, which blocks the primer binding site.

PCR suppression induces multiplication of DNA fragments limited by both restrictive sites or two short adapters. A limited number of PCR products enables their effective analysis in agarose or polyacrylamide gel stained with ethidium bromide.

A well-selected set of restrictive ndonucleases and adaptors allows their application for testing DNA of related species.

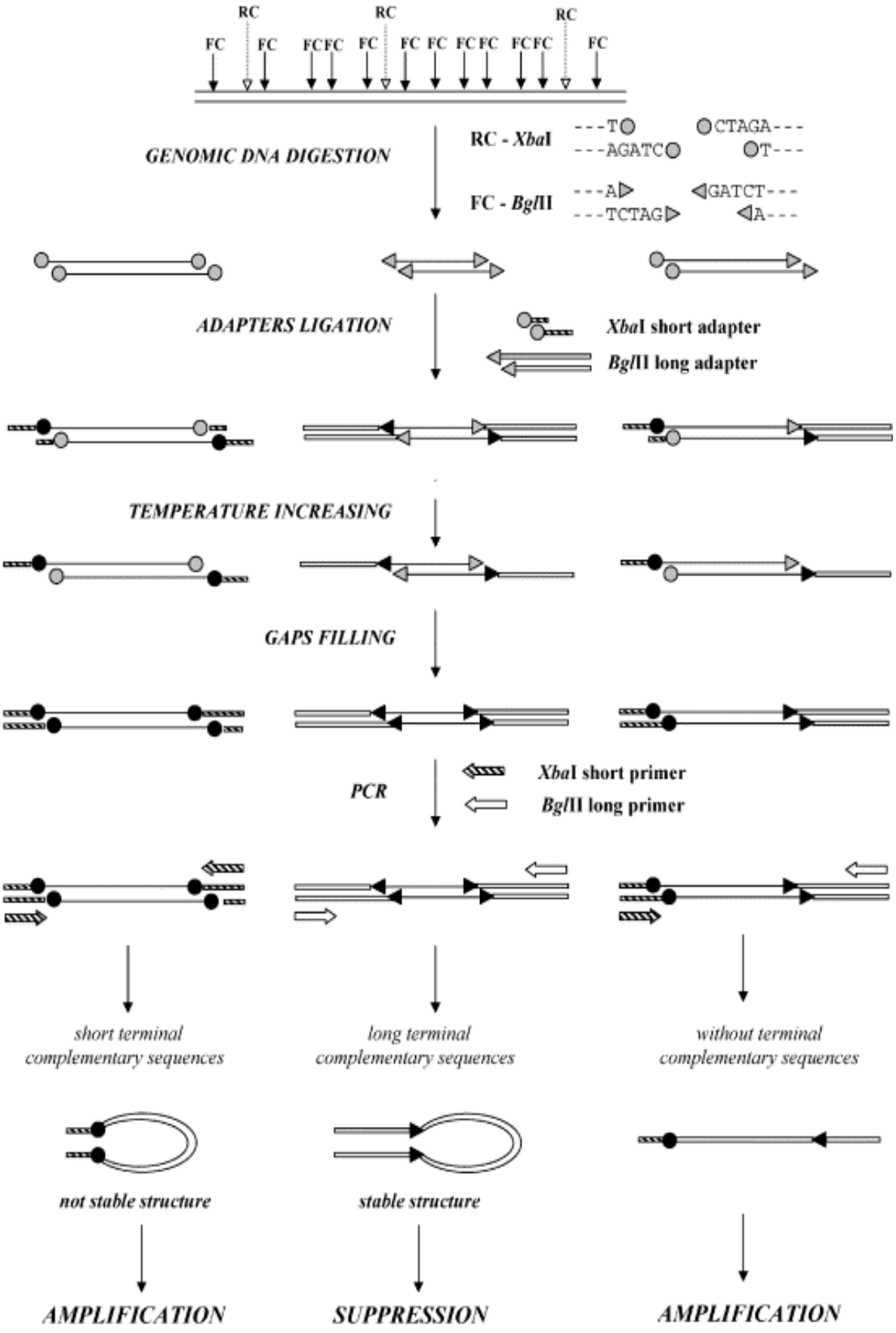

Figure1. ADSRRS method scheme-fingerprinting, originally according to Krawczyk et al. [16]

RESULTS

In the study, 50 strains of *Escherichia coli* isolated from the material collected from patients hospitalized in internal diseases, gynaecological, and surgical clinics as well as the Intensive Care Unit of the teaching hospital. Most samples were collected from internal diseases clinics – 32% and neonatology – 30%. As far as the systems are concerned, the most numerous samples were collected from the digestive system (26%) and the urinary system (22%). Other samples were collected from the female reproductive tract, the hearing organ, body fluids, drains and respiratory tubes and the respiratory system.

The algorithm UPGMA (unweighted pair group method with arithmetic mean) was used for the analysis of the results of strains gene-typing using the ADSRRS method. It allowed the comparison of the genetic similarity of the examined strains. Based on the dendrogram, nine clones, from A to I, were distinguished. Two clones, A and B, were determined significant and most numerous (38% and 26%, respectively), giving total 64% of all analysed strains.

Clone A appeared most frequently in the internal diseases clinics (37%) and comprised nearly 44% of all E.coli strains isolated from those departments. It was 3.5-fold higher value of the coefficient of the appearance of the strains of this clone as compared to clone B in the material from all the departments.

There were 26% of E.coli strains of clone An isolated from the material from the neonatological clinics, which comprised 33.3% of total population of bacteria of clone A.

The participation of strains isolated from samples collected in the gynecological clinic in the general population of clone A equaled 15% and comprised 37.5% of all strains from that particular department.

Further 15% of total population of bacteria of clone A were isolated from diagnostic material delivered from the intensive care unit. They constituted a half of all *Escherichia coli* strains isolated from that department.

Assuming place of appearance as the analysis criterion – the physiological systems and drains – it was stated that bacteria belonging to clone A were most numerous among strains isolated from the respiratory system and the urinary system (32% and 21% of the population of clone A, respectively). It constituted 66% and 36% of all *E. coli* strains isolated from that places.

Clone B The highest percentage was observed in the material from the neonatological clinic (54%), which is 47% of all *E. coli* strains isolated from that department. It was 1.5-fold higher than the number of strains of clone A and 3.5-fold more than that of clone D.

Next 15% of the strains comprising clone B originated from diagnostic material from the gynaecological clinic. It was 25% of E.coli isolated from that place.

From the pool of strains classified as clone B, 15% were observed in samples from the internal diseases clinics and comprised 12% of all isolated from that place E.coli strains.

Strains classified as clone B were most frequently isolated from the material from the digestive system (38%) and constituted 38% of all analysed strains collected from the diagnostic material.

The degree of genetic similarity of clones A, B, D, E, and F was analysed, and it was shown to be not smaller than 90%. The highest degree of strains homology was observed in the range of clones F (93.3%) and B (92%) while the lowest degree of affinity to all analysed strains was presented in the case of clone I and equaled approximately 84.3%.

During the examination of clones A, B, and G, the occurrence of E.coli strains was observed, with identical patterns of stripes in the electrophoresis of the PCR products. The affinity concerned strains in the range of specific clones and thus strains mentioned above were recognized identical.

Most homologous strains (7 out of 11 examined) were collected from the neonatological clinic; the rest was isolated from the internal diseases clinic and the gynaecological clinic. The most numerous were clone B.

Statistical analysis

The data were analysed statistically using the Chi-square test based on STATISTICA StatSoft. (StatSoft Polska, Kraków, Poland). The results of $p<0.05$ were considered statistically significant. The statistical examination of differences in clones A and B occurrence in the material from the respiratory system and the digestive system showed the values at the border of significance ($p=0.057$).

DISCUSSION

Bacteria classified as one species can differ as far as the phenotype is concerned. Genotypes of specific strains can present even bigger changeability.

Diversity can concern both quality and quantity of genetic material contained in the bacterial cell. According to Bergthorsson and Ochman, the difference in the length of genomic DNA in the case of *Escherichia coli* may reach even 1Mb [17]. It comprises approximately 20-25% of the mean size of the genome of this species [18]. In the bacterial cell, besides „chromosome" material additional elements like plasmides or bacteriophages may also occur [19].

The aim of the study was to compare the genetic similarity of 50 randomly chosen *Escherichia coli* strains isolated from diagnostic material from patients hospitalized in the University Teaching Hospital in Białystok.

There are various experimental methods to compare genetic material, including RAPD, REA-PFGE, RFLP, SSCP, AFLP, SSH, PCR-M and ADSRRS. We chose the ADSRRS method as its essence is the limitation of the number of replicated fragments of analysed DNA to the level that allows its analysis using electrophoresis and observation in the UV light. It is characterized by stronger discrimination and repetitiveness with markedly lower cost than PFGE or AFLP [20].

There are studies in the literature that used the ADSRRS method for genotyping of clinically significant species of bacteria, namely *Enterococcus faecium*, *Serratia marcescens, Klebsiella pneumoniae, Staphylococcus aureus* isolated from people [21-24].

However, there are no studies that examined *Escherichia coli*, which is one of the most crucial pathogen among strains that are responsible for hospital infections [20].

In Krawczyk et al. reports, the ADSRRS method was used simultaneously with PFGE as the golden standard for molecular diversification method. On the other hand, the study that evaluated affinity of Staphylococcus aureus strains used PCR-MP as a comparative method [22,23].

That technique was also developed by Masny and Płucienniczak [13]. In the case of Enterococcus faecium, Krawczyk et al. showed that the ADSRRS method allowed to distinguish nine clones and PFGE – 8 clones.

All the cases revealed that the strength of discrimination of the ADSRRS (as well as PCR-MP) was not smaller than PFGE and the time of analyses was markedly shorter. An additional advantage of the ADSRRS method is the fact that there is no need to possess specialistic equipment for pulsed-field electrophoresis.

An infection that develops in a patient during his stay in the hospital and is not a cause of his hospitalization is determined as in-hospital infection [25].

Nowadays, in-hospital infections become a crucial problem, not only clinical but also economic. The stay in hospital is prolonged, the patient requires additional medicines and procedures, which increases social costs and influences negatively on patient's quality of life.

The literature indicates that E.coli strains are responsible for approximately 10% of in-hospital infections [26] although Binczycka-Anholcer et al. showed even 15%. The presence of bacterium of a given species is determined by, among others, the kind of material and the place the sample comes from.

According to the literature, *Escherichia coli*, is the most frequently isolated bacterium from samples of the urinary system. Giedrys-Kalemba showed that the participation of *E. coli* in in-hospital infections of the urinary system comprised from 40 to 60% [27]. The results are also confirmed by own studies, where in the monitored period the presence of *Escherichia coli* was confirmed in 40% of samples from the urinary system.

Own studies also confirmed that over 75% of E.coli strains come from material collected in the internal diseases departments, which was confirmed by the literature [16,28].

Own studies showed the presence of 9 clones using the ADSRRS method in randomly chosen E.coli strains. The presence of 2 dominant clones, denoted A and B /own symbols/, was stated. The dominant clones comprised 64% of examined strains.

The highest number of clone A (47%) was isolated from the internal diseases departments as the highest number of patients is treated in the internal diseases departments.

The second of most frequent and dominant clones B came from the neonatological departments and gynaecological departments. It can result from the transfer of an infection from mother to child and simultaneously from the inmature immune system of the infant. The data are confirmed in the literature [29].

In the case of infant infection, the most important are a time limit of etiologic factor determination. Then, molecular methods of identification of microorganisms are extremely useful.

CONCLUSIONS

The results indicate the significant role of monitoring of homogeneity of bacteria strains isolated in the range of the health care providers. It is directly connected with the safety of hospitalized patients and the effectiveness and course of treatment. The use of the ADRSSR method gives the opportunity of early determination of colonization of the monitored place.

Conflicts of interest

The authors declare no conflicts of interest

REFERENCES

1. Escherich T. Die Darmbacterien des Neugeborenen und Säuglings. Fortschr Med. 1885;3:515-22.
2. Schulze J, Schiemann M, Sonnenborn U. 120 years of *E. coli*. Its importance in research and medicine. Hagen, Germany: Alfred-Nissle-Gesellschaft e.V. 2006
3. Szewczyk EM. Diagnostyka bakteriolo-giczna. Warszawa. Wydawnictwo Naukowe PWN; 2005. p.111-116,129-31 (Poilsh).
4. NCBI. Taxonomy database. [Internet]. [cited 2017 Apr 20]. Available from: http:// www.ncbi.nlm. nih.gov/Taxonomy/ Browser/ wwwtax.cgi??mode=Info&id=561.
5. Madappa T, Go CH. *Escherichia coli* Infections [Internet]. [cited 2017 Apr 20]. Availabe from:

http://emedicine.medsca pe.com/article/217485-overview.
6. Todar`s Online Textbook of Bacteriology. Todar K. Pathogenic *E. coli*. [Internet]. [cited 2016 Apr 20]. Available from: http://www.textbooko fbacteriology.net/e.coli.html.
7. Wijetunge DS, Gongati S, DebRoy C, Kim KS, Couraud PO, Romero IA, Weksler B, Kariyawasam S. Characterizing the pathotype of neonatal meningitis causing *Escherichia coli* (NMEC). BMC Micro biol. 2015 Oct;14:15:211. doi: 10.1186/s12866-015-0547-9.
8. Rendón MA, Saldaña Z, Erdem AL, Monteiro-Neto V, Vázquez A, Kaper JB, Puente JL, Girón JA. Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization. PNAS 2007 Jun; 104 (25): 10637–42.
9. Martinez-Medina M, Garcia-Gil LJ. *Escherichia coli* in chronic inflammatory bowel diseases: An update on adherent invasive *Escherichia coli* pathogenicity. World J Gastrointest Pathophysiol. 2014 Aug;5(3):213–27.
10. Wieler LH, Tigges M, Ebel F, Schäferkordt S, Djafari S, Schlapp T, Baljer G, Chakraborty T. The enterohemolysin phenotype of bovine Shiga-like toxin-producing *Escherichia coli* (SLTEC) is encoded by the EHEC-hemolysin gene. Vet Microbiol. 1996 Sep;52(1-2):153-64.
11. Masny A, Plucienniczak A. Fingerprinting of bacterial genomes by amplification of DNA fragments surrounding rare restriction sites. Biotechniques. 2001 Oct;31(4):930-4.
12. Boom R, Sol C, Beld M, Weel J, Goudsmit J, Wertheim-van Dillen P. Improved silica-guanidiniumthiocyanate DNA isolation procedure based on selective binding of bovine alpha-casein to silica particles. J Clin Microbiol. 1999 Mar;37(3):615-9.
13. Masny A, Płucienniczak A. Ligation mediated PCR performed at low denaturation temperatures--PCR melting profiles. Nucleic Acids Res. 2003 Sep 15;31(18):e114.
14. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory Manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor; 1989. p.1.52-1.73.
15. Sambrook J, Russell DW. Molecular cloning: A laboratory Manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor; 2001. p. A8.20 – A8.21.
16. Krawczyk B, Lewandowski K, Bronk M, Samet A, Myjak P, Kur J. Evaluation of a novel method based on amplification of DNA fragments surrounding rare restriction sites (ADSRRS fingerprinting) for typing strains of vancomycin-resistant Enterococcus faecium. J Microbiol Methods. 2003 Mar;52(3):341-51.
17. Bergthorsson U, Ochman H. Distribution of chromosome length variation in natural isolates of *Escherichia coli*. Mol Biol Evol. 1998 Jan;15(1):6-16.
18. Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K, Han CG, Ohtsubo E, Nakayama K, Murata T, Tanaka M, Tobe T, Iida T, Takami H, Honda T, Sasakawa C, Ogasawara N, Yasunaga T, Kuhara S, Shiba T, Hattori M, Shinagawa H. Complete genome seque nce of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res. 2001 Feb 28;8(1):11-22.
19. Iguchi A, Thomson NR, Ogura Y, Ogura Y, Saunders D, Ooka T, Henderson IR, Harris D, Asadulghani M, Kurokawa K, Dean P, Kenny B, Quail MA, Thurston S, Dougan G, Hayashi T, Parkhill J, Frankel G Complete genome sequence and compa rative genome analysis of enteropatho genic *Escherichia coli* O127:H6 strain E2348/69. J Bacteriol. 2009 Jan;191(1): 347-54.
20. Krawczyk B. Diagnostyka molekularna w zakażeniach szpitalnych. Post. Mikrobiol. 2007;46(4):367-78 (Polish).
21. Dziekiewicz-Mrugasiewicz M, Rzewuska M, Stefańska I, Jakubczak A. Select pathogenic characteristics, as well as the genotypic diversity of Staphylococcus aureus, strains isolated from milk of cows with mastitis in the north-east region of Poland. Medycyna Wet. 2008; 64(8):1012-5. (Polish).
22. Krawczyk B, Leibner J, Barańska-Rybak W, et al. ADSRRS-fingerprinting and PCR MP techniques for studies of intraspecies genetic relatedness in Staphylococcus aureus. J Microbiol Methods. 2007 Nov;71(2):114-22.
23. Krawczyk B, Naumiuk L, Lewandowski K, Baraniak A, Gniadkowski M, Samet A, Kur J. Evaluation and comparison of random amplification of polymorphic DNA, pulsed-field gel electrophoresis and ADSRRS-fingerprinting for typing Serra tia marcescens outbreaks. FEMS Immunol Med Microbiol. 2003 Oct 15;38(3): 241-8.
24. Krawczyk B, Samet A, Czarniak E, Szczapa J, Kur J. Extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a neonatal unit: control of an outbreak using a new ADSRRS technique. Pol J Microbiol. 2005; 54(2):105-10.
25. Dzierżanowska D, Jeliaszewicz J. Zakażenia szpitalne. Bielsko-Biała: α-Medica Press; 1999. p.396–406 (Polish).
26. Sierocka A, Cianciara M. Monitorowanie zakażeń szpitalnych. Probl. Hig. Epidemiol. 2010; 91(2):323-8 (Polish).
27. Giedrys-Kalemba S, Jursa J. Zakażenia dróg moczowych na oddziałach urologicznych. Przegl Urol. 2007;8/4(44) [Internet]. [cited 2016-12-01]. Available from: http://www.przeglad-urologiczny.pl/ arty kul.php?1303 (Polish).

28. Filius PM, Gyssens IC, Kershof IM, Roovers PJ, Ott A, Vulto AG, Verbrugh HA, Endtz HP. Colonization and resistance dynamics of gram-negative bacteria in patients during and after hospitalization. Antimicrob Agents Chemother. 2005 Jul;49(7):2879-86.
29. Sohn AH, Garrett DO, Sinkowitz-Cochran RL, Grohskopf LA, Levine GL, Stover BH, Siegel JD, Jarvis WR. Prevalance of nosocominal infections in neonatal intensive care units: results from the first national point-prevalence survey, J Pediatr 2001 Dec;139(6):821–7.