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Biofilm Formation by Clinical *Klebsiella* Strains Expressing Various Types of Adhesins on Catheters Made of Different Materials

Tworzenie biofilmu przez kliniczne szczepy *Klebsiella* wytwarzające różne typy adhezyn na cewnikach wykonanych z różnych biomateriałów

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Abstract

Background. *Klebsiella* bacilli are frequent causes of nosocomial infections associated with the use of urinary (CAUTI) and intravenous catheters (CR-BSI) due to their ability to form biofilm on biomaterials. The adhesive properties of *Klebsiella* bacilli connected with the presence of fimbrial and non-fimbrial adhesins play a very important role in the pathogenicity of these bacteria.
Objectives. Investigation of the correlation between the pathogenic properties of the clinical *Klebsiella* strains (the capsule, adhesin types), the type of biomaterial and the degree of *in vitro* biofilm formation on catheters.
Material and Methods. Sixty-nine clinical *Klebsiella* strains isolated from patients hospitalised in different hospital wards were tested using hemagglutination test for fimbriae occurrence and Richard's method and electron microscopy for biofilm formation.
Results. Level of adhesion of the clinical *Klebsiella* strains to biomaterials was associated with the catheter material. Electron microscope images showed various biofilm structures produced by different *Klebsiella* strains.
Conclusions. There exists a direct connection between the degree of biofilm formation by the tested *Klebsiella* strains and the chemical composition of the catheter. Surprisingly, it seems there is no direct correlation between the ability of strain to biofilm formation and the type of fimbriae expressed by the strain. In light of own research, strains which do not express fimbriae are able to form biofilm as well (**Adv Clin Exp Med 2010, 19, 4, 443–453**).

Key words: *Klebsiella,* adhesins, biofilm formation, biomaterials.

Streszczenie

Wprowadzenie. Pałeczki z rodzaju *Klebsiella* często są odpowiedzialne za infekcje szpitalne związane z zakażeniami cewników moczowych (CAUTI) oraz żylnych (CR-BSI) dzięki zdolności do tworzenia biofilmu na biomateriałach. Zdolności adhezyjne pałeczek *Klebsiella* są związane z ekspresją na ich powierzchni fimbrialnych i niefimbrialnych adhezyn będących istotnymi czynnikami chorobotwórczości tych bakterii.
Cel pracy. Zbadanie korelacji między wybranymi czynnikami chorobotwórczości szczepów *Klebsiella* (otoczka, typ adhezyn), typem biomateriału a stopniem tworzenia biofilmu *in vitro* na cewnikach.
Materiał i metody. 69 szczepów *Klebsiella* wyizolowanych z zakażeń od pacjentów hospitalizowanych na różnych oddziałach szpitalnych zbadano testem hemaglutynacji w celu oznaczenia typu fimbrii oraz testem Richardsa i metodą mikroskopii elektronowej w celu oceny tworzenia struktur biofilmu.
Wyniki. Poziom adhezji klinicznych szczepów *Klebsiella* do biomateriałów zależy od typu chemicznego materiału tworzącego cewnik. Użycie mikroskopii elektronowej pozwoliło na zaobserwowanie różnych struktur biofilmu tworzonych przez różne szczepy *Klebsiella.*
Wnioski. Istnieje ścisły związek między zdolnością do tworzenia biofilmu przez badane szczepy *Klebsiella* a składem chemicznym cewnika. Zastanawiającym wynikiem jest brak bezpośredniej korelacji między zdolnością szczepu do formowania biofilmu a typem fimbrii wytwarzanych przez szczep. W świetle badań własnych także szczepy niewytwarzające fimbrii są w stanie wytworzyć biofilm na powierzchni biomateriału (**Adv Clin Exp Med 2010, 19, 4, 443–453**).

Słowa kluczowe: *Klebsiella,* adhezyny, tworzenie biofilmu, biomateriały.

Infections in hospitalised patients in the treatment of whom various biomaterials (catheters, drainage tubes, vascular prostheses, joint prostheses, etc.) are used for diagnostic and treatment purposes have been a serious and yet unresolved problem of clinical medicine. The use of the above materials poses a threat of infecting the organism of the patient with nosocomial bacteria [1] or with bacteria forming the microflora of the skin, mucous membrane and intestines of the patient. *Staphylococci* and gram-negative bacteria such as *E. coli, Klebsiella* and *Pseudomonas* adhere to synthetic materials forming a biofilm on their surface. They spread to the patient's urine or bloodstream through the catheter lumen and along its external surface. The type of material from which the catheter is made plays a significant role in the pathogenesis of colonization and infection. The most serious complication connected with using urinary and venous catheters are catheter-related infections (CRI) diagnosed in about 250,000 patients in American hospitals, including catheter-related bloodstream infections (CR-BSI) and catheter-associated urinary tract infections (CA-UTI). According to *National Nosocomial Infections Surveillance* (NNIS), catheter-related septicaemia is the third most frequent nosocomial infection type. It extends the hospitalization period and increases its cost by US$3,000–40,000 per patient and mortality rate by 10–20 per cent. According to European data, vascular catheters are the cause of 23.5–66 per cent of bacteraemia cases [2, 3]. Catheter-related urinary tract infections are among the most frequent nosocomial infections. The above infections are diagnosed each year in the United States in more than a million hospital patients. CAUTI may also be the primary cause of bloodstream infections referred to as urosepsis which is characterized by a high mortality rate. The above infections are most often caused by gram-negative *Escherichia coli, Klebsiella, Proteus*, *Pseudomonas, Enterobacter,* and *Serratia* [4–6].

Klebsiella have many pathogenic properties which enable their survival and spreading in the hospital environment as well as their adhesion to synthetic surfaces. The pathogenicity of *Klebsiella* strains is caused by some of its cell elements such as fimbriae, non-fimbrial adhesion factors, capsules, lipopolysaccharide [2, 8], as well as extracellular exotoxins (enterotoxins or cytotoxins) [7]. *Klebsiella* resistance to many antibiotics and chemotherapeutic agents (ESBL and AmpC enzymes, carbapenemases: KPC and MBL) has grown significantly in recent years which increased the risk of nosocomial infections caused by these bacteria [9, 10]. The production of various types of adhesins and the ability to form a biofilm on the surface of biomaterials play an important role in the colonisation and survival of the microorganisms on the mucous membranes of the macroorganism and on synthetic surfaces of catheters and implants. Shortly after the implantation, the synthetic material which most often displays hydrophobic properties is covered with proteins and glycoproteins of the host as well as by other cells. Relatively smooth and flat surfaces of implants are colonized by one or two layers of macrophages, while rougher materials are covered by a larger number of both macrophages and giant cells [11, 12]. Research conducted by many authors suggests a significant role played by urine components in the bacterial adhesion process. Among them there are urea, creatinine, Tamm-Horsfall protein, mannosides, hormones and urine pH. Some of them promote and some inhibit bacterial growth, e.g. Tamm-Horsfall protein covers uroepithelium and binds type 1 adhesins of gram-negative bacteria such as *Klebsiella*. The mechanism of biofilm formation is currently the subject of many research projects. Biofilm can be defined as an aggregate of bacteria set in an organic polymer matrix adhering to some surface [15, 17]. Bacterial adhesion to surfaces can be divided into clear phases: non-specific adhesion (reversible), specific adhesion (irreversible) and the formation of the proper biofilm [12]. The biofilm formation process is affected by many variables. Among others, it depends on bacteria species, type of surface to which the bacteria will adhere, environmental factors and on the products of some genes. Bacterial adhesion is initiated by electrostatic bonds, hydrophobic interactions and Van der Waals forces between the bacterial surface, the surrounding liquid and the surface of the synthetic material (non-specific adhesion) [16]. If the bacteria express fimbriae or other surface exposed structures, the interaction with the surface can take place via the "bridges" formed. Other elements of the adhesion process include extracellular proteins and bacterial polysaccharides demonstrating the ability to bind to the surface of the synthetic material or to the biofilm covering it [11]. Facilitated bacterial aggregation and the formation of microcolonies on the synthetic material surface are the final effect of the adhesion [12]. The second phase of adhesion is anchoring and the use of molecular mediators between the specific adhesins and the biomaterial surface. During the above process, a binding substance is produced (exopolysaccharide) that binds the biomaterial surface with the specific ligands located on the fimbriae. At this point, adhesion becomes irreversible as the microorganisms tightly adhere to the surface [11]. Biofilm formation is regulated by means of chemical signals referred to as "quorum sensing". It is a kind of communication between bacterial cells effected through the secretion of signalling compounds. The bacteria

start to form a kind of community [18]. The thick and complex biofilm growth starts from an active replication and formation of additional cell components by attaching bacteria reacting with organic and inorganic molecules in the environment thus forming the glycocalyx. Biofilm formation process differs in time and depends first of all on the bacteria species, type of biomaterial used and the time of exposure to the plankton cell suspension. It is known that the biofilm structure and its physiological properties make bacterial cells resistant to antibacterial agents such as antibiotics and disinfectants which is most probably a result of delayed penetration through the biofilm structure and of the changed metabolism of the microorganisms [13]. On the other hand, there are few unambiguous studies concerning the role in the biofilm formation process of fimbrial and nonfimbrial adhesins and other pathogenic factors such as capsules.

With regard to the role of *Klebsiella* in catheter-related infections, the purpose of the paper was to study the *in vitro* formation of biofilm by clinical and standard *Klebsiella* strains on catheters made of different materials. Because of the participation of fimbriae in biofilm formation, the purpose of the research was to determine the correlation between the type of adhesins produced by the strain and the intensity of cell aggregate formation on the catheter surface. The paper also aims at defining the role of the capsule in adhesion to synthetic surfaces and in biofilm formation and shows electron microscope images of biofilm structures formed by *Klebsiella* strains on catheter surfaces.

Material and Methods

Bacterial Strains

Sixty-nine clinical *Klebsiella* strains isolated from patients hospitalised in different hospital wards were tested. 30 strains were isolated from urinary tract infections UTI, 23 from blood and 16 from other clinical samples. The investigation covered strains producing several distinct types of adhesins (fimbria MS, MR and P-like nonfimbrial adhesins). In the study, 7 *Kl. pneumoniae* reference strains of determined K and O antigens from the Ørskov Collection in Copenhagen and their unencapsulated variants obtained using specific bacteriophages, were used.

Tested Catheters

In the tests, the following urinary catheters were used – Nelaton made of polyvinyl chloride, Foley made of latex, Foley (all-silicone) and the Cavafix polyurethane venous catheter.

Hemagglutination Test

The phenotypic activity of fimbriae MR, MS and P-like adhesins, was evaluated using the hemagglutination method according to Duguid et al. [14] with 3% suspension of bovine, guinea pig or human $0P_1$ erythrocytes in the presence or absence of D-mannose.

Assessment of Biofilm Formation

In vitro biofilm formation was evaluated using the Richard's method [1]. In this assay, soluble colourless TTC (2,3,5 triphenyltetrazolium chloride) was reduced to insoluble red formazan by electron transfer associated with active oxidative bacterial metabolism and was precipitated intracellularly. Catheters were cut up into 1 cm pieces and gas-sterilized. 1 McFarland suspension obtained from original initial bacterial cultures was incubated with catheters fragments. After 24-hour incubation, the biomaterials were washed 3-times in NaCl and transferred to 5 ml fresh TSB broth. Thereafter, 20 µl of 1% colourless TTC solution was added to each of the tubes and after 24-hour incubation the red formazan formation was detected. A catheter fragment incubated in TSB without a bacterial suspension was used as a negative control. The influence of the capsule on the ability to form biofilm was estimated using unencapsulated mutants and their encapsulated variants.

Electron Microscopy

The Richard's assay was validated by electron microscopy. The catheters with TTC reduced on its surface were examined in JSM-5800 LV Jeol microscope with a magnification of 3000×. The presence of the characteristic biofilm structure on catheters was observed [15]. The microscopy procedure was proceed in the following manner: The rinsed and dried catheter was fixed using 3% glutarate for 15 min in the room temperature. Thereafter, the catheter was rinsed twice with phosphate buffer in purpose of fixative elimination. Next step was dehydratation in increasing concentrations of ethanol (25, 60, 95, 100%) for 5 minutes in every solution. After rinsing off the ethanol, the samples were dried. Finally, the catheters were covered with gold.

Results

All investigated strains showed the ability to form biofilm on catheters after 24 hours incubation. For most of the strains, the value of formazan reduction was determined as average or strong. The investigated strains exhibited the highest degree of biofilm formation on venous catheters from polyurethane and on urinary catheters from polyvinyl chloride. The lowest adhesion rate was observed for all-silicone Foley catheters. The encapsulated strains exhibited slightly lower biofilm formation ability than the unencapsulated strains. The presence of the characteristic biofilm structure on catheters was confirmed in electron microscopy.

When determining the degree of adhesion to catheter surfaces in the tested strains it was discovered that only few *Klebsiella* strains did not have the biomaterial adhesion and biofilm formation ability. The majority of the strains demonstrated such ability after 24 hours of incubation. The level of TTC reduction to red formazan was evaluated thus determining the amount of biofilm formed by the strain. The biggest number of strains demonstrated an average ability to form biofilm on catheters made of different materials, while some strains were very effective in their biofilm formation (+++). The degree of catheter staining interpreted as the degree of biofilm formation is presented in Fig. 1.

Catheter surface photographs which were taken with an electron microscope show the usefulness of the Richards method in the evaluation of adhesion and biofilm formation by the *Klebsiella* strains. The electron microscope images of all the strains causing a high degree of catheter staining included characteristic biofilm structures. Electron microscope images of negative controls and

Fig. 1. The Richards's method – the level of colorless TTC reduction into the red-colored formazan on the catheter surface correlates with level of biofilm formation. The level of TTC reduction was estimated according to the following criteria: [–] lack of colour on catheter, [+] catheter surface is getting slightly pink; [++] redness of the whole surface; [+++] redness of the surface and the inside of the catheter; [++++] redness of the whole catheter, cloudiness and the red medium

Ryc. 1. Metoda Richardsa – poziom redukcji bezbarwnego TTC do czerwonego formazanu na powierzchni cewnika koreluje z poziomem utworzenia biofilmu. Poziom redukcji TTC oceniano następująco: [–] brak zabarwienia cewnika [+] powierzchnia cewnika jest lekko zabarwiona na różowo; [++] cała powierzchnia zewnętrzna cewnika jest zabarwiona na czerwono; [+++] cewnik jest zabarwiony na czerwono na powierzchni wewnętrznej i zewnętrznej; [++++] cewnik jest zabarwiony na powierzchni zewnętrznej i wewnętrznej, widoczne zmętnienie i zaczerwienienie pożywki

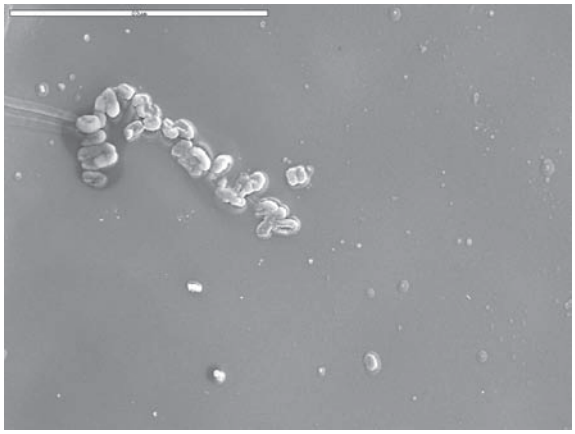

Fig. 2. The first step of biofilm formation on the surface of Cavafix venous catheter after 4-hour incubation (*Klebsiella* strain isolated from blood*)* – bacterial aggregation. JSM-5800 LV Jeol microscope with a magnification of 3000×

Ryc. 2. Pierwszy etap formowania się biofilmu na powierzchni cewnika żylnego Cavafix – po 4 godzinnej inkubacji (szczep *Klebsiella* izolowany z krwi) jest widoczna agregacja bakteryjna. Mikroskop JSM-5800 LV Jeol powiększenie 3000×

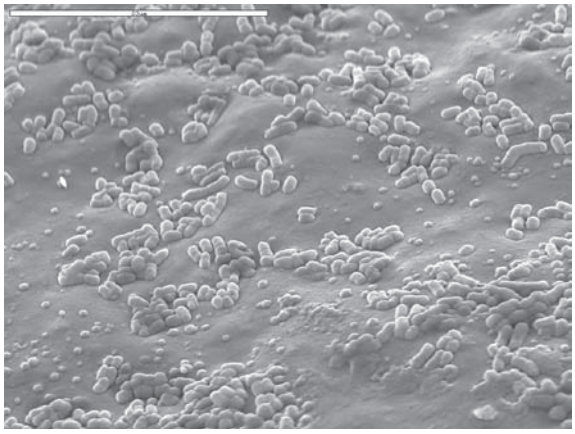

Fig. 3. The second step after 8-hour incubation (the same strain). JSM-5800 LV Jeol microscope with a magnification of 3000×

Ryc. 3. Drugi etap po 8-godzinnej inkubacji (ten sam szczep). Mikroskop JSM-5800 LV Jeol, powiększenie 3000×

of strains with a negative test result obtained using the Richards method did not show any biofilm formed. It was also found that different strains formed biofilm with different structures. Net-like, aggregate type structures connected by an extracellular substance were observed. Figs. 2–9 include examples of biofilm structures produced by the tested strains on catheter surfaces.

Figs. 10-12 confirm the visible dependence between the biofilm formation ability and the chemical composition of the biomaterial. The tested *Klebsiella* strains produced the biggest amounts of biofilm on catheters made of polyurethane and polyvinyl chloride (PVC). The phenomenon is especially evident for strains isolated from blood where most of the strains displayed strong adhesion to polyurethane and there was not a single strain that would not produce biofilm on that material.

However, the majority of encapsulated reference strains demonstrate slightly lower adhesion to

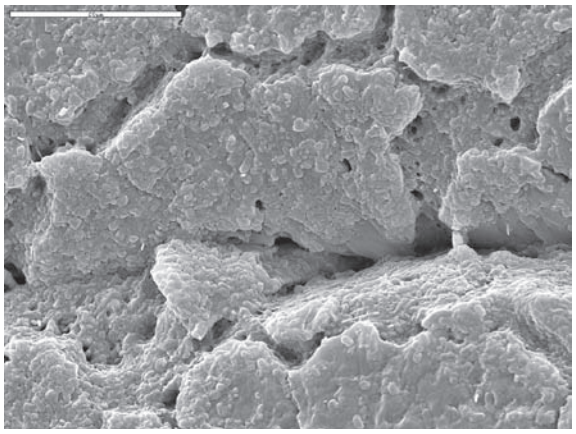

Fig. 4. Biofilm structure on the surface of a Cavafix venous catheter after 24-hour incubation (the same strain isolated from blood). JSM-5800 LV Jeol microscope with a magnification of 3000×

Ryc. 4. Struktura biofilmu utworzonego na powierzchni żylnego cewnika Cavafix po 24-godzinnej inkubacji (ten sam szczep izolowany z krwi). Mikroskop JSM-5800 LV Jeol, powiększenie 3000×

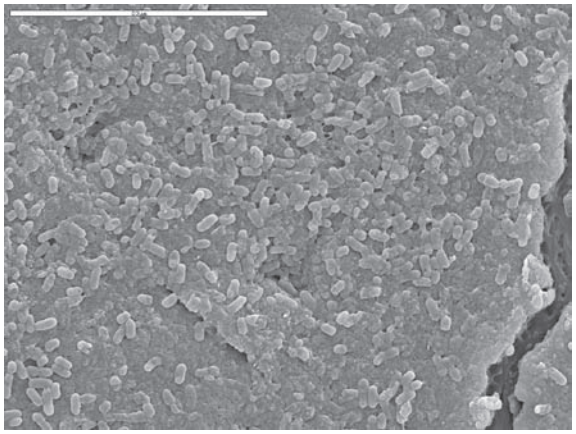

Fig. 5. Biofilm structure on the surface of a Foley catheter after 24-hour incubation (the strain isolated from urine). JSM-5800 LV Jeol microscope with a magnification of 3000×

Ryc. 5. Struktura biofilmu utworzonego na powierzchni cewnika Foleya po 24-godzinnej inkubacji (szczep izolowany z moczu). Mikroskop JSM-5800 LV Jeol, powiększenie 3000×

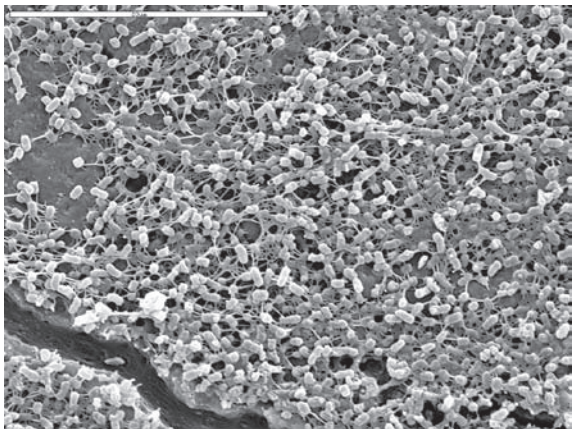

Fig. 6. Biofilm structure formed by *Klebsiella* on a Nelaton catheter (strain isolated from UTI) after 24-hour incubation. The strain produced a lot of extracellular polysaccharide creating filaments joining the bacterial cells. JSM-5800 LV Jeol microscope with a magnification of 3000×

Ryc. 6. Struktura biofilmu utworzonego przez *Klebsiella* na cewniku Nelaton (szczep izolowany z z.u.m.) po 24 godzinnej inkubacji. Szczep wytwarza dużą ilość pozakomórkowego polisacharydu tworzącego filamenty łączące komórki bakteryjne. Mikroskop JSM-5800 LV Jeol, powiększenie 3000×

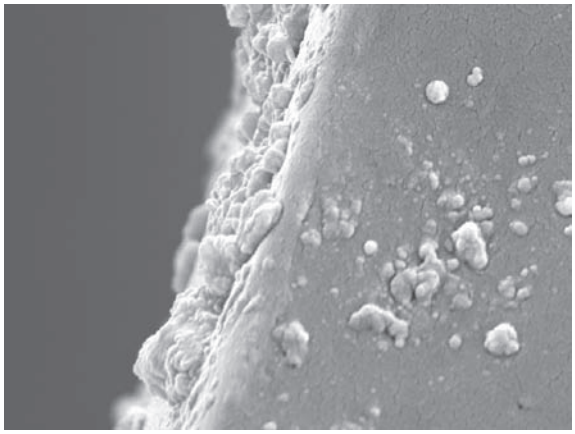

Fig. 7. Biofilm structure after 24-hour incubation – longitudinal section of a Cavafix venous catheter (strain isolated from blood). JSM-5800 LV Jeol microscope with a magnification of 3000×

Ryc. 7. Struktura biofilmu utworzonego po 24-godzinnej inkubacji – przekrój wzdłużny cewnika żylnego Cavafix (szczep izolowany z krwi). Mikroskop JSM-5800 LV Jeol, powiększenie 3000×

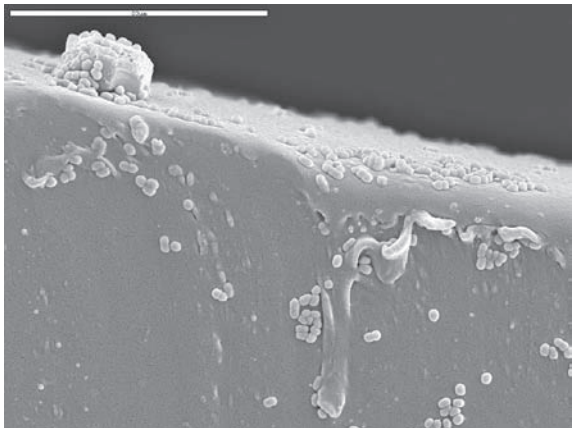

Fig. 8. Biofilm structure after 24-hour incubation – cross-section of a Foley catheter (unencapsulated strain) JSM-5800 LV Jeol microscope with a magnification of 3000×

Ryc. 8. Struktura biofilmu po 24-godzinnej inkubacji – przekrój poprzeczny cewnika Foleya (szczep bezotoczkowy). Mikroskop JSM-5800 LV Jeol, powiększenie 3000×

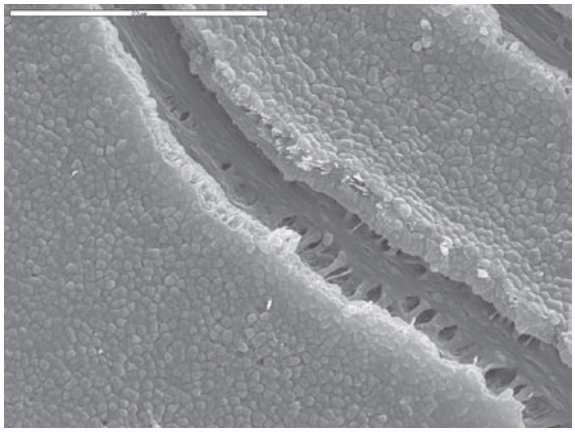

Fig. 9. Biofilm structure after 24-hour incubation – vertical section of a Nelaton PVC catheter (strain isolated from the gastrointestinal tract). JSM-5800 LV Jeol microscope with a magnification of 3000×

Ryc. 9. Struktura biofilmu po 24-godzinnej inkubacji – przekrój pionowy cewnika Nelaton wykonanego z PVC (szczep izolowany z przewodu pokarmowego). Mikroskop JSM-5800 LV Jeol, powiększenie 3000×

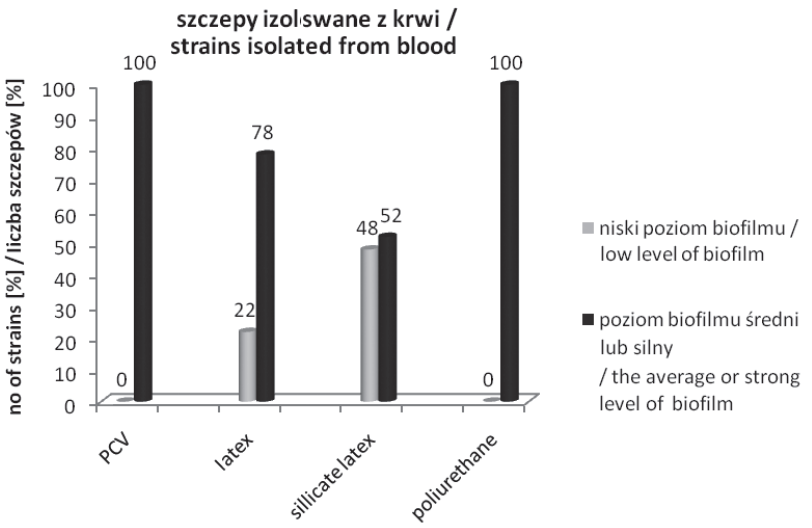

Fig. 10. Level of biofilm formation on tested catheters – *Klebsiella* strains isolated from blood

Ryc. 10. Poziom tworzenia biofilmu na badanych cewnikach przez szczepy *Klebsiella* izolowane z krwi

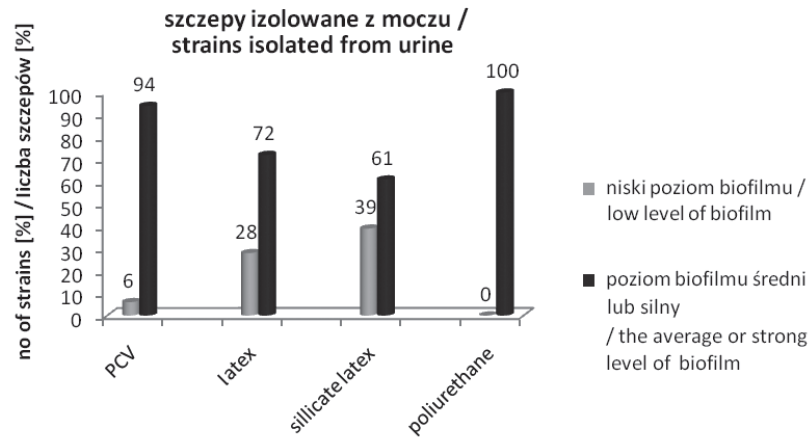

Fig. 11. Level of biofilm formation on tested catheters – *Klebsiella* strains isolated from urine

Ryc. 11. Poziom tworzenia biofilmu na badanych cewnikach przez szczepy *Klebsiella* izolowane z moczu

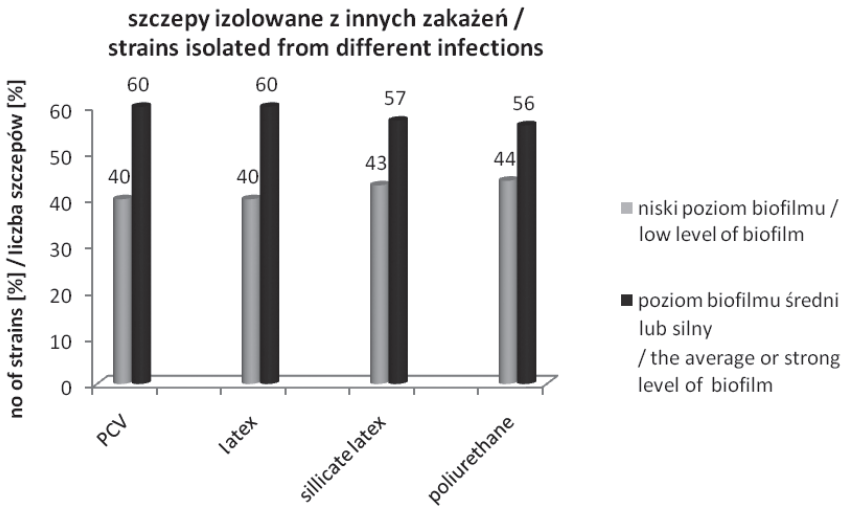

Fig. 12. Level of biofilm formation on tested catheters – *Klebsiella* strains isolated from different infections

Ryc. 12. Poziom tworzenia biofilmu na badanych cewnikach przez szczepy *Klebsiella* izolowane z innych zakażeń

Table 1. Biofilm formation by reference encapsulated *Klebsiella* strains and by their unencapsulated variants

Tabela 1. Tworzenie biofilmu przez referencyjne otoczkowe szczepy K*lebsiella* i ich warianty bezotoczkowe

Strain no (Nr szczepu)	Fimbriae type (Typ fimbrii)	Level of adhesion to: (Poziom adhezji do:)			
		PVC	latex	silicone latex	polyuretane
59 K+	3	++	+	+	+
59 K–	3	+++	+	+	+++
57 K+	3	+/–	+/–	–	+
57 K–	3	+	+	+/–	+++
67 K+	3	+++	++	–	+++
67 K–	3	++	–	+++	++
114 K+	1,3	+	+/–	+/–	++
114 K–	1,3	++	+	–	++
42 K+	3	+	+	+	+++
42 K–	3	++	+++	+	++++
64 K+	3	+	+/–	–	+
64 K–	3	+	+/–	–	+
16 K+	3	++	+	++	+++
16 K–	3	+++	+++	+++	++++

K+ – encapsulated strain. K– – unencapsulated strain.
K+ – szczep otoczkowy. K– – szczep bezotoczkowy.

biomaterials comparing to their uncapsulated variants. Although the number of investigated strains was statistically insignificant above mentioned differences were clearly visible among encapsulated and uncapsulated variants of strain. The analysis of individual strains adhesion showed that regardless of catheter type and capsule type, the uncapsulated variant of the strain demonstrated stronger adhesion than the encapsulated strain (Tab. 1).

No direct relationship (statistically significant) was found between the type of fimbriae expressed by the given strain and the ability to form biofilm on the catheter surface. The strains with different types of fimbria formed the biofilm on the comparable level, depending only on the chemical type of the biomaterials that were When evaluating biofilm formation ability of reference *Klebsiella* strains and of their unencapsulated variants it was

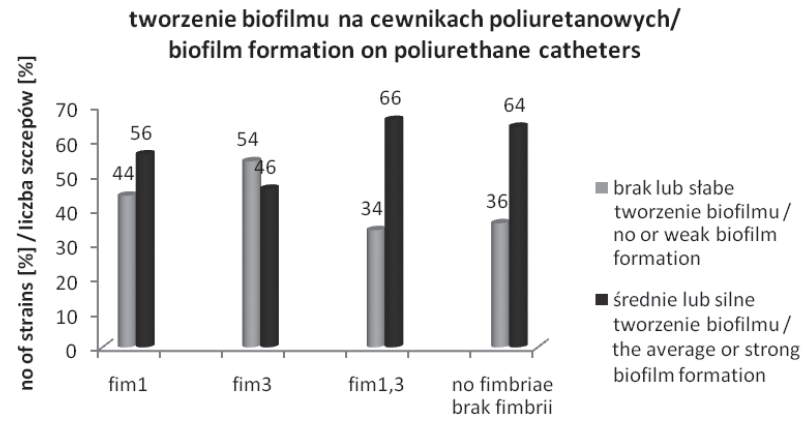

Fig. 13. Relationship between expressed type of fimbriae and ability of biofilm formation on the polyurethane catheters by tested *Klebsiella* strains

Ryc. 13. Zależność między typem fimbrii wytwarzanych przez badane szczepy *Klebsiella* a zdolnością do tworzenia biofilmu na cewnikach poliuretanowych

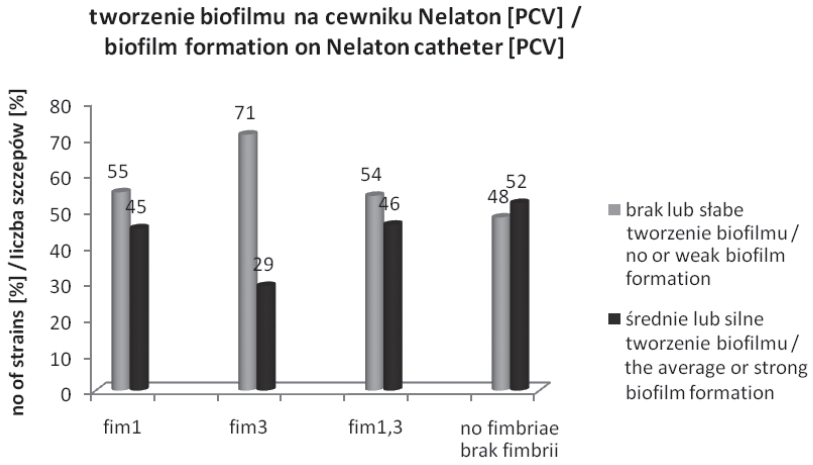

Fig. 14. Relationship between expressed type of fimbriae and ability of biofilm formation on the Nelaton catheters by tested *Klebsiella* strains

Ryc. 14. Zależność między typem fimbrii wytwarzanych przez badane szczepy *Klebsiella* a zdolnością do tworzenia biofilmu na cewnikach typu Nelaton

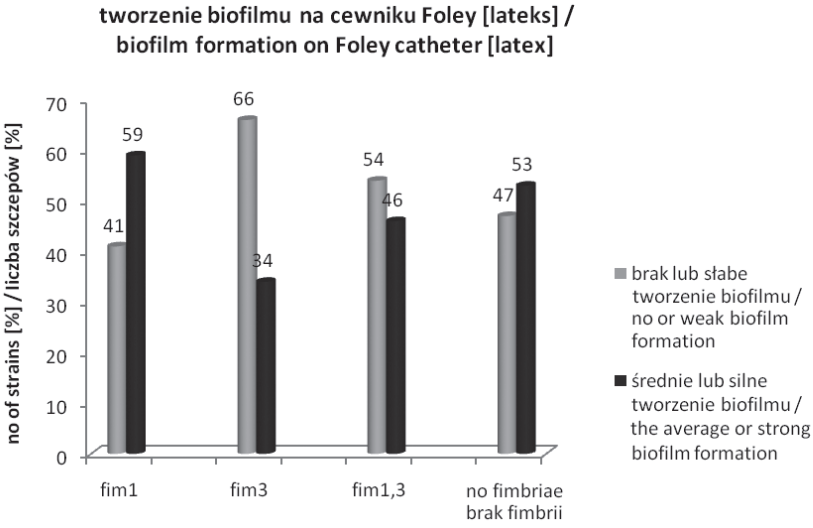

Fig. 15. Relationship between expressed type of fimbriae and ability of biofilm formation on Foley catheters by tested *Klebsiella* strains

Ryc. 15. Zależność między typem fimbrii wytwarzanych przez badane szczepy *Klebsiella* a zdolnością do tworzenia biofilmu na cewnikach Foleya

discovered that majority of the strains had biofilm used (Figs. 13–16).

Discussion

Venous catheters inserted to central and peripheral veins as well as urinary catheters are currently the most frequent risk factor for hospitalised patients. Their more and more popular application is the reason behind the increased frequency of catheter-related nosocomial infections. The risk of catheter-related infection is connected first of all with the time during which the catheter remains inserted and it is estimated that each day the risk increases by 5%. Another important factor is the

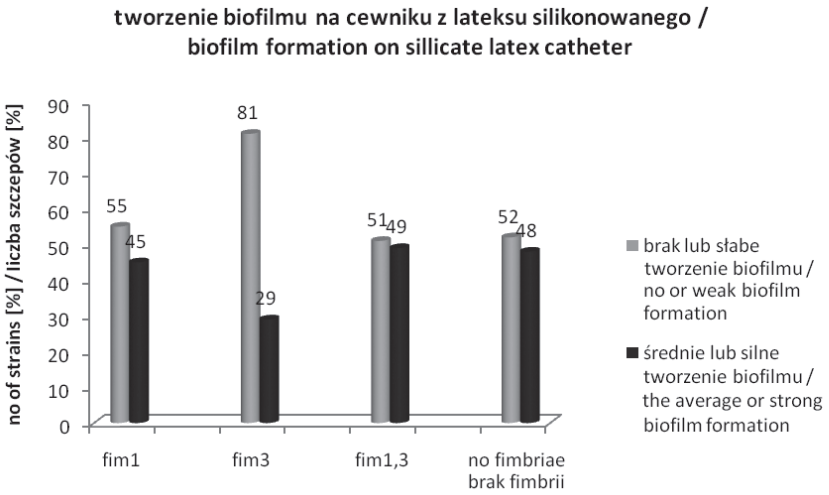

Fig. 16. Relationship between expressed type of fimbriae and ability of biofilm formation on silicate latex catheters by tested *Klebsiella* strains

Ryc. 16. Zależność między typem fimbrii wytwarzanych przez badane szczepy *Klebsiella* a zdolnością do tworzenia biofilmu na silikonowanych cewnikach lateksowych

type of catheter and the chemical composition of the material it is made of [16].

The increased frequency of isolating *Klebsiella* as the etiological factor of catheter-related urinary tract infections inspired a growing interest in some pathogenicity mechanisms of the bacteria such as adhesion or biofilm formation.

In the case of coagulase-negative staphylococci, mature biofilm can be formed *in vitro* on the catheter surface as soon as after 2–4 hours [19, 20]. The tests conducted show that for the *Klebsiella* strains the process takes slightly longer. Fully formed biofilm was observed in the electron microscope after 18–24 hours of bacterial suspension activity on the catheter surface. Figs. 2–4 show biofilm formation phases after 4, 8 and 24 hours. In none of the tested strains TTC reduction was observed visually after 4 hours and the electron microscope image showed single aggregates (microcolonies) on the catheter surface, forming the first stages of biofilm formation. In the majority of cases, 24 hours were sufficient for the characteristic biofilm structure to form on the biomaterial surface (Figs. 5–9).

The images obtained from the photographs taken using the electron microscope confirmed the usefulness of the Richards method in the evaluation of the degree to which *Klebsiella* strains form biofilm on various catheter types. In each cases of positive Richard's test the structure of biofilm were observed in scanning microscope. Control photographs (catheter incubated with NaCl) and photographs of catheters marked as strongly colonized have confirmed that the reduction of colourless triphenyltetrazolium chloride (TTC) to red formazan takes place only with the participation of living microorganisms.

It seems however that the structure of the biofilm formed depends on the strain. In some of the tested *Klebsiella* strains, the authors have observed tangled structures resembling a cobweb, most probably built of extracellular polysaccharide (Fig. 6). It would be interesting to obtain this substance, analyze its composition and compare it with similar substances found in other biofilm-forming bacteria. In many strains, the biofilm structure looks as if it was embedded in the catheter or forming protruding aggregates (Figs. 4 and 5).

The expression by *Klebsiella* bacilli of various fimbriae and non-fimbrial adhesins is undoubtedly not irrelevant in the process of biofilm formation. In particular, MR fimbriae (type 3) are regarded as likely to participate in the process, owing to their ability to adhere to the cells of various epithelia, including the bladder epithelium [21–23]. As opposed to environmental strains, among strains isolated from clinical material, bacilli completely devoid of fimbriae were isolated only sporadically [21, 24]. Strains expressing only type 1 fimbriae were isolated relatively less frequently [25]. The above is reflected in the profile of the *Klebsiella* strains used for tests for the purposes of this paper. Only about 20% of the tested strains did not express type 3 fimbriae (only type 1 fimbriae) and 10 % did not express any type of fimbria. Only one strain (No 912) was completely devoid of fimbrial adhesins (the structures were not observed in the electron microscope). When analyzing the link between the adhesin type found in the *Klebsiella* strains and the degree of biofilm formation on the catheter surface, no direct correlation with any particular type was found. It turned out that both the strains having P-like adhesins, type 3 fimbriae, type 1 fimbriae or several types at the same time, had similar biomaterial adhesion properties (Tab. 1, Figs. 13–16). The *Klebsiella* strains that did not express any type of fimbria, formed the biofilm structures with the comparable level to the strains with various type of fimbria. Observed differences depend only on the chemical type of catheter (the highest level of biofilm formation on

polyurethane catheters) (Figs. 10-12). Above results stay in contrast to research of Langstraat et al. [29] what prompt to the conclusion that influence of different types of adhesins on the process of biofilm formation requires further investigation.

The above confirms the argument that the biofilm formation process is a complex phenomenon, dependent on many factors and on the signals transmitted (quorum sensing) [18].

What seems very important is the fact that among the tested *Klebsiella* strains none was found which would be totally devoid of the ability to adhere to biomaterial surfaces. Even strain 912, devoid of fimbriae and treated for the purposes of this paper as a negative control, exhibited an average level of adhesion to the venous catheter and low adhesion to urinary catheters. It means that the *Klebsiella* bacilli are among the microorganisms that have strongly developed mechanisms allowing them to effectively colonize catheters and to form biofilm structures, and consequently to cause catheter-related infections [26].

It was very interesting to analyze biofilm formation ability of encapsulated and unencapsulated strains. The authors have compared the degree of adhesion to standard catheters made of various materials exhibited by encapsulated strains and by their unencapsulated variants obtained in previous tests. Own observation was that in each case the unencapsulated mutants exhibited stronger adhesion than the parental encapsulated strain (Tab. 1) It is strange to the extent that the capsule is regarded to be one of the pathogenicity factors, although its role in biofilm formation has not been proven. Thus, the capsule which performs an important role in protecting the bacteria against phagocytosis, can simultaneously, owing to its hydrophobicity, inhibit or reduce biofilm aggregate formation on catheter surfaces. Due to difficulties with obtaining unencapsulated mutants of the *Klebsiella* strains, the number of tested strains was not large. Therefore, an unambiguous evaluation of the phenomenon requires further detailed studies.

A direct connection was observed between the degree of biofilm formation by the tested *Klebsiella* strains and the chemical composition of the catheter used in the experiment. All the tested strains exhibited the strongest adherence to polyurethane from which Cavafix venous were made and to PVC in Nelaton urinary catheters. Especially in the case of venous catheters, there were practically no strains which would exhibit a lack of adhesion to polyurethane, which seems especially dangerous and confirms the role of biofilm in the development of serious septic infections related to the colonization of vascular catheters by the *Klebsiella* strains. The tested strains exhibited the lowest adhesion to urinary catheter made of siliconized latex. In the above case it was discovered that a significant percentage of strains did not form any biofilm at all on the biomaterial surface or that such biofilm formation was very weak. Differences in adhesion resulting from the chemical composition of the catheter used are confirmed in research findings of other authors.

In the experimental research papers published using the Richards method, very similar results were obtained with respect to *Staphylococcus* [19, 20] and *Pseudomonas* [27] strains. It is not however a rule. Various bacteria species can probably prefer a certain chemical composition of the biomaterial to which their adhesion is the strongest. Similar experiments conducted with the *Enterococcus* species [28] produced contrary conclusions – the strains adhesion was the strongest to the Foley catheter made of siliconized latex and the weakest to the Nelaton catheter made of PVC. This paper confirmed the ability of the clinical *Klebsiella* strains to form biofilm structures on the surfaces of catheters widely used in medicine. A direct link was also demonstrated between the chemical composition of the biomaterial and the intensiveness of biofilm formation. However, the tests conducted did not give an unambiguous answer to the question concerning the degree to which fimbrial adhesins participate in the initiation and formation of biofilm. Research of the bacterial community and biofilm formation mechanisms exhibited by *Klebsiella* have started to develop rapidly only recently, so there still remains a lot to be discovered. Biofilm formation is undoubtedly a complex phenomenon and it is affected by many factors, both on the side of the bacteria and the macroorganism.

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