**Introduction**

Coagulase-negative staphylococci (CNS) constitute the main part of the human skin microbiome. For this reason, their role as pathogens was underestimated and their identification did not include a distinction between species on a regular basis (Eng et al., 1982). Amongst the genus *Staphylococcus*, only coagulase-positive *Staphylococcus aureus* was considered as pathogenic and thoroughly analysed in many studies. It was not until the late 1960s that one of the CNS, *Staphylococcus saprophyticus*, was associated with frequent urinary tract infections (John et al., 1978). Later, in the 1970s, the first CNS infections in patients with invasive and indwelling medical devices were reported (Liekweg & Greenfield, 1977; Rupp & Archer, 1994).

Diagnostic protocols designed by Kloos & Schleifer (1975) and the later introduction of molecular techniques enabled a more accurate identification of the already known staphylococci species as well as new species (Kloos & Schleifer, 1975). Differences in the frequency of isolation from clinical materials, and in the virulence factors and antimicrobial susceptibility amongst staphylococci species were also noted (Corse & Williams, 1968; Gemmell & Dawson, 1982; John et al., 1978). Simultaneously, increasing numbers of CNS infections were observed as a result of progress in medicine. According to a study conducted in the USA between 1980 and 1989, CNS contribution to hospital-acquired bacteraemia increased from 9 to 27% (Schaberg et al., 1991).

Phylogenetically, staphylococci constitute a very coherent group. *S. aureus* versus *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* shows ~75% average nucleotide identity values, which proves their close genetic relatedness (Konstantinidis et al., 2006; Lamers et al., 2012). That fact is also indicated in the topology of a dendrogram based on the 16S rRNA sequence in *Bergey’s Manual of Systematic Bacteriology* (Vos et al., 2009) as well as in whole-genome sequencing projects comparing different staphylococci strains (Takeuchi et al., 2005) and 134 isolates of *S. haemolyticus* from geographically diverse origins (Cavanagh et al., 2014). Nowadays, *S. aureus*, *S. epidermidis* and *S. haemolyticus* are the most frequent aetiological agents of staphylococcal infections (Takeuchi et al., 2005). However, their virulence factors differ depending on the species. Moreover, the diversity between particular strains also seems to be...
essential. *S. aureus*, the most virulent species, causes a broad spectrum of infections, from minor skin diseases to systemic infections and sometimes septic shock (Otto, 2012). *S. epidermidis*, due to its ability to produce extracellular slime substances and proteins binding extracellular matrix, can easily form biofilms (Arciola et al., 2002; Rosenstein & Götz, 2013). The ability to form a biofilm was also associated with the existence of biofilm-associated protein Bap, encoded by the *bap* gene (Tormo et al., 2005). *S. epidermidis* is predominant in blood infections, particularly in patients with artificial heart valves or with intravenous catheters commonly used in hospitals (Liekweg & Greenfield, 1977; Rupp & Archer, 1994). In contrast to *S. aureus*, *S. haemolyticus* is associated with immune-compromised patients and patients with implanted medical devices (Silva et al., 2013). Most *S. haemolyticus* strains seem to lack the important virulence attributes. However, some enzymes, cytolysins or surface substances are indicated in the literature as factors contributing to its virulence (Daniel et al., 2014; Flahaut et al., 2008; Simango, 2005), but none of them was identified as a crucial and determinative factor (Fredheim et al., 2009). Despite this, *S. haemolyticus* is, after *S. epidermidis*, the second most frequently isolated CNS from clinical cases, particularly from blood infections, including sepsis (Becker et al., 2014; Klingenberg et al., 2007; Liakopoulou et al., 2008; Silva et al., 2013). Therefore, what is the reason for the increasing clinical significance of *S. haemolyticus*? The most important factor might be the facility to acquire multiresistance against available antimicrobial agents. In a recent study, Barros et al. (2012) noted that 75% of analysed *S. haemolyticus* isolates displayed multiresistance. This species also plays an important role in the dissemination of resistance genes, contributing to the emergence of epidemic clones of a more virulent nosocomial pathogen, *S. aureus* (Cavanagh et al., 2014; Fluit et al., 2013).

**Multiresistance**

The increasing number of CNS in hospital-acquired infections (HAIs) is closely related to their antimicrobial resistance and the ability to survive in a hospital environment. In the late 1980s, many studies were conducted in order to determine the scale of the problem and the sources of multiresistance. The antimicrobial resistance profiles of CNS have been monitored. Archer & Climo (1994) indicated that the percentage of CNS resistant to meticillin, oxacillin and nafticillin amongst HAI strains increased from 20 to 60% between 1980 and 1989, according to The National Nosocomial Infection Survey, which included data collected from selected hospitals in the USA. An even larger increase was reported at university hospitals in the state of Iowa, where the frequency of bacteraemia caused by CNS increased from 5.2 to 42 cases per 10 000 admissions from 1984 to 1987 (Martin et al., 1989). Simultaneously, the widest spectrum of antimicrobial resistance amongst CNS was observed in *S. haemolyticus* strains. During the next decades, multiresistance of *S. haemolyticus* was reported with an increasing frequency. Most of the analysed strains were resistant to the commonly used antiseptic agents and antibiotics, notably more frequently than strains of any other species amongst CNS (Cavanagh et al., 2014; Hope et al., 2008). Many authors reported *S. haemolyticus* strains as resistant to one or more antibiotics amongst penicillins, cephalosporins, macrolides, tetracyclines, quinolones, aminoglycosides, glycopeptides and fosfomycines (Holden et al., 2013; Lebeaux et al., 2012; Shittu et al., 2004). In 2008, strains resistant to the majority of available antimicrobial agents were described for the first time (Campanile et al., 2008). Multiresistant *S. haemolyticus* strains spread in the hospital environment (Cavanagh et al., 2014; Rodriguez-Aranda et al., 2009). Ternes et al. (2013) showed that 55.9% of infants carried multiresistant CNS in their nasal cavity. Amongst them the most frequently isolated species were *S. haemolyticus* (38.3%) and *S. epidermidis* (38.0%).

Multiresistant strains of *S. haemolyticus* also pose a serious problem in animal pathology. They have been isolated both from ruminants (Vanderhaeghen et al., 2014) and domestic animals (Ruzauskas et al., 2014). Due to the possibility of transmission between animals, their owners and veterinary staff, animals can act as reservoirs of multidrug-resistant strains of *S. haemolyticus* (Lloyd, 2007).

The scale of the phenomenon of the increasing resistance amongst *S. haemolyticus* strains for the most frequently used antimicrobials (β-lactams, macrolides, aminoglycosides and quinolones) over the years is presented in Table 1.

**Meticillin-resistant *S. haemolyticus* (MRSH)**

Meticillin resistance became the reason for a significant limitation in the use of β-lactam antibiotics. Some authors even describe the present times as the ‘post-penicillin age’. *S. aureus* strains have been reported to be resistant to penicillin since its discovery and introduction to the general population, due to their ability to produce β-lactamases. In the 1950s, *S. aureus* strains producing penicillinase became widespread (Chambers, 1988). In 1959, meticillin – a semisynthetic, β-lactamase resistant antibiotic – was introduced to clinical use. At that time, natural resistance of *S. aureus* strains to meticillin was not observed and the problem of penicillin resistance seemed to be permanently solved (Lebeaux et al., 2012; Sutherland & Rolinson, 1964). Nevertheless, strains with reduced susceptibility to meticillin were reported shortly after the introduction of meticillin (Sutherland & Rolinson, 1964). In 1961, the first strain of meticillin-resistant CNS was isolated in a clinical laboratory in the UK (Stewart, 1961). However, at that time, CNS were not regarded as particularly pathogenic to humans. Resistance to meticillin, described in the above-mentioned study, was substantially higher in CNS than in *S. aureus*.
The observed MIC for meticillin in CNS reached 2000 µg ml⁻¹, in comparison with the maximum of 20 µg ml⁻¹ for *S. aureus*. In the following years, meticillin-resistant *S. aureus* (MRSA) strains were isolated in other European countries, as well as in the USA, Japan and Australia (Enright *et al.*, 2002).

It is well known that the mechanism of meticillin resistance determines the resistance to all β-lactam antibiotics: penicillins, cephalosporins, carbapenems and monobactams (Kollef, 2009; Widmer, 2008). In *S. aureus* (Hiramatsu *et al.*, 2002), which is the subject of the majority of studies on meticillin resistance, as well as in CNS (Bochniarcz *et al.*, 2013), this mechanism is associated with the presence of the *mecA* gene, encoding the modified transpeptidase penicillin-binding protein PBP2a, which is natively responsible for the synthesis of pentaglycine bridges in peptidoglycan. Despite differences in the peptidoglycan structure between *S. haemolyticus* and *S. aureus*, its similarity is sufficient to develop the same resistance mechanism (Billot-Klein *et al.*, 1996). Analysis of *mecA* gene sequences in GenBank reference strains of *S. aureus, S. haemolyticus* and *S. epidermidis* showed 99.95% similarity, which proves the theory of the interspecies transfer of *mecA* gene. β-Lactam agents bind native PBP2 protein and block its activity, whereas they exhibit a much lower affinity to the modified PBP2a protein (John & Harvin, 2007). In the 1970s, meticillin resistance was more frequent amongst meticillin-resistant CNS than amongst MRSA. This phenomenon is still current (John & Harvin, 2007). Unfortunately, even in current papers, the species of the investigated CNS groups are often not specified, which hinders the possibility of evaluating the contribution of *S. haemolyticus* to the phenomenon of meticillin resistance.

Another important feature of staphylococci is their ability to survive in the hospital environment. According to a study conducted in South Korea in 2011, MRSH strains were found on 51.4% of X-ray cassettes in a Radiology Department. PFGE analysis showed a genetic similarity between the isolated strains, which indicates their clonal spread in the hospital environment via medical devices (Kim *et al.*, 2012). Such an occurrence was also reported previously in other studies (Degener *et al.*, 1994; Tabe *et al.*, 1998), which showed that patients and hospital staff might also be a reservoir for multiresistant *S. haemolyticus* strains (Perl *et al.*, 1999; Rahman *et al.*, 2012; Squeri *et al.*, 2012).

Molecular analysis demonstrated that *mecA* genes, part of SCCmec cassettes, are associated with meticillin resistance. These cassettes are mobile genetic elements which might be horizontally transferred. (Mlynarczyk & Mlynarczyk, 2008; Zong *et al.*, 2011). SCCmec cassettes are localized in the genomic bacterial DNA, at the 3’ end of the orfX gene encoding ribosomal methyltransferase. Apart from the mec gene complex (*mecA, mecR1, mecl*), they comprise the ccr gene complex encoding chromosomal recombinase, which enables the integration of cassette and chromosomal DNA, and sometimes also other genes encoding virulence factors or associated with resistance to various antimicrobial agents (Hanssen & Ericson Sollid, 2006; Ito *et al.*, 2001). SCCmec cassettes consist of 10–60 kbp (International Working Group on the Classification of

Table 1. *S. haemolyticus* resistance to selected antimicrobials according to various studies since 1989

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<tr>
<td>Penicillin</td>
<td>ND</td>
<td>87.5</td>
<td>84.4</td>
<td>95.0</td>
<td>ND</td>
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<tr>
<td>Meticillin</td>
<td>80.0</td>
<td>72.3</td>
<td>70.5</td>
<td>ND</td>
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<tr>
<td>Gentamicin</td>
<td>79.0</td>
<td>67.9</td>
<td>58.4</td>
<td>73.0</td>
<td>92.9</td>
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<tr>
<td>Erythromycin</td>
<td>79.0</td>
<td>78.8</td>
<td>75.1</td>
<td>64.0</td>
<td>85.7</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>75.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>28.6</td>
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<tr>
<td>Trimethoprim</td>
<td>63.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>57.1</td>
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<td>Chloramphenicol</td>
<td>11.0</td>
<td>ND</td>
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<td>25.0</td>
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<td>Ciprofloxacin</td>
<td>ND</td>
<td>54.9</td>
<td>54.9</td>
<td>ND</td>
<td>92.9</td>
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<tr>
<td>Clindamycin</td>
<td>2.7</td>
<td>4.6</td>
<td>ND</td>
<td>47.0</td>
<td>ND</td>
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<tr>
<td>Fusidic acid</td>
<td>ND</td>
<td>31.0</td>
<td>30.6</td>
<td>ND</td>
<td>50.0</td>
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<tr>
<td>Teicoplanin</td>
<td>ND</td>
<td>11.4</td>
<td>12.7</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Vancomycin</td>
<td>ND</td>
<td>0.0</td>
<td>0.0</td>
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*ND, No data included in the study.
†N=70 *S. haemolyticus* isolates from 60 patients in the USA, n=51 hospitalized, strains isolated mainly from wounds, urine and blood; phenotypic analysis (MIC) (Froggatt *et al.*, 1989).
‡N=184 *S. haemolyticus* isolates from hospitals in the UK; phenotypic analysis (MIC) (Andrews *et al.*, 2000).
¶N=173 *S. haemolyticus* isolates from hospitals in the UK; phenotypic analysis (MIC) (Andrews *et al.*, 2000).
§N=64 *S. haemolyticus* isolates from hospitalized patients in Rio de Janeiro, Brazil; phenotypic analysis (disc diffusion assay) (Barros *et al.*, 2012).
||N=14 *S. haemolyticus* isolates from European countries (Belgium, n=2; Germany, n=10; Spain, n=2); genotypic analysis (Cavanagh *et al.*, 2014).
Staphylococcal Cassette Chromosome Elements, 2009; Shore & Coleman, 2013). At present, 11 types of SCCmec cassettes and many subtypes have been identified in S. aureus strains. However, due to ongoing research and continuously increasing knowledge in this field, this list will certainly be extended in the near future. What is more, it is sometimes impossible to associate the cassette’s type to that described in the literature (International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements, 2009), especially during the analysis of SCCmec cassettes in CNS strains. The largest diversity of SCCmec sequences is observed amongst S. epidermidis, S. haemolyticus and Staphylococcus hominis strains. In these cases, entire cassette sequencing is often required in order to determine the similarity to those described in the literature (Takeuchi et al., 2005).

The most frequently identified SCCmec cassette type in S. haemolyticus strains is type V. Types IV and VII have also been reported. Although these cassettes are present in a relatively small number of strains, S. haemolyticus is indicated to be the reservoir of SCCmec elements for other staphylococci, due to its facility to transfer genes to other species (Fluit et al., 2013; Szczuka & Kaznowski, 2014). At the neonatal intensive care unit in Örebro University Hospital in Sweden in 2008, a case of SCCmec type V cassette transfer from MRSH to meticillin-susceptible S. aureus was reported (Berglund & Söderquist, 2008). The study conducted by Ternes et al. (2013) indicated that the prevalence of SCCmec amongst CNS is high and that hospitalization increases the prevalence significantly. Whilst 60% of CNS strains isolated from patients’ physiological microbiome at the moment of admission to the hospital possessed the mecA gene, the percentage of mecA-positive CNS strains at the moment of discharge from the hospital increased to 83.6%.

**Resistance of S. haemolyticus to the last-chance antimicrobial drugs**

As a consequence of the increasing resistance to meticillin, the search for new antimicrobial drugs designed to treat infections caused by meticillin-resistant strains was commenced. A new class of antibiotics, glycopeptides (e.g. vancomycin introduced into the clinic in 1958 and teicoplanin introduced into the clinic 1984), inhibits the synthesis of cell walls by interfering with peptidoglycan synthesis (Berglund & Söderquist, 2008; Srinivasan et al., 2002). Resistance to glycopeptides results from the acquisition and expression of operons that substitute a terminal D-lactate or D-serine for the D-alanine, which reduces the vancomycin-binding affinity (Rice, 2012). Another mechanism of glycopeptide resistance involves a vanA operon contained on a transposon residing on a conjugal plasmid. However, this mechanism has been mostly described for the genus Enterococcus (Qureshi et al., 2014).

In the early 1980s, CNS were regarded as genetically unable to develop resistance to vancomycin (Veach et al., 1990). Staphylococci strains resistant to glycopeptides were not reported during the first 20 years following its introduction into clinical use, (Srinivasan et al., 2002). Resistance to vancomycin and teicoplanin is still quite rare amongst staphylococci, but many studies have indicated that S. haemolyticus might play an important role in this phenomenon. S. haemolyticus was the first Gram-positive bacteria reported as being resistant to glycopeptides, before any other staphylococci and enterococci (Kristóf et al., 2011). This species has been also suggested as being more active than other CNS in generating clones with increased glycopeptide (especially teicoplanin) MICs (Bia-vasco et al., 2000).

In 1987, the first case of peritonitis caused by S. haemolyticus with reduced susceptibility to vancomycin in a dialysis patient was reported (Schwalbe et al., 1987). Amongst CNS, it was S. haemolyticus that was documented as being resistant to teicoplanin for the first time (Biavasco et al., 2000). It was suggested that S. haemolyticus is unique amongst CNS and especially predisposed to resistance to glycopeptides, because of its extraordinary genome plasticity and tendency to frequent DNA rearrangements (Takeuchi et al., 2005). According to various studies, the percentage of hospital-isolated S. haemolyticus strains with reduced susceptibility to teicoplanin is increasing (Bannerman et al., 1991; Cercenado et al., 1996; Goldstein et al., 1990). What is alarming is that the strains were also isolated from infants in neonatal intensive care units (Perreira et al., 2014). At the University Hospital in Leiden in The Netherlands, the susceptibility of CNS to teicoplanin was compared over the years. Between 1985 and 1994, the percentage of CNS strains with reduced susceptibility to teicoplanin increased from 2 to 20% (Sloos et al., 1998). The data show that the rapidity of the selection process for teicoplanin-resistant strains is very high. The case of a teicoplanin-resistant strain isolation from a Hickmann catheter was reported after the first course of treatment with this antibiotic (Cunningham et al., 1997). The most alarming cases are associated with infections caused by CNS strains resistant to both teicoplanin and vancomycin (Fajardo Olivares et al., 2011; Sieradzki et al., 1998). They also emerged after long-term treatment with teicoplanin in infections caused by S. epidermidis strains (Aubert et al., 1990; Blans & Troelstra, 2001). The wide use of glycopeptides in the hospital environment led to the selection of strains with reduced susceptibility to these antibiotics, with MIC values $\sim 4–8$ mg l$^{-1}$ for vancomycin and/or 8–16 mg l$^{-1}$ for teicoplanin (Nakipoglu et al., 2005; Natoli et al., 2009; Tabe et al., 2001). The first cases like these were reported in 1979 and 1983, when CNS were regarded as bacteria with a low pathogenic potential (Siebert et al., 1979; Tuazon & Miller, 1983). Although the increase in resistance to glycopeptides in S. aureus strains is suggested by some authors to be connected with the phenomenon of MIC creep (incremental increases in glycopeptide MICs for strains over time), it is believed that such...
a phenomenon has not occurred in CNS (Ahlstrand et al., 2011).

Infections caused by multiresistant S. haemolyticus strains might be associated with implantations and sometimes the only way to prevent a systemic infection is to remove an implant (Daniel et al., 2014). As a rule, in the case of infections caused by staphylococci resistant to glycopeptides, treatment should be based on linezolid – a synthetic chemotherapeutic of the oxazolidinones drug class (Perry & Jarvis, 2001). Linezolid blocks protein synthesis by binding to 23S rRNA and preventing the creation of the translation initiation complex (Livermore, 2003; Long & Vester, 2012; Quiles-Melero et al., 2013). Resistance to linezolid amongst CNS is currently negligible; however, the first cases of S. haemolyticus strains resistant to linezolid have been reported in India and in various countries in Europe (Gupta et al., 2012; Mazzariol et al., 2012; Mendes et al., 2014a, b, c; Rajan et al., 2014; Tarazona et al., 2007). Recent research performed in Italy on S. haemolyticus strains showed that resistance to linezolid is associated with a G2576T mutation (Mazzariol et al., 2012), which leads to linezolid’s reduced affinity for the ribosome. The same mutation has been described previously for S. aureus (Tsiodras et al., 2001). A double mutation of the rRNA gene (C2190T and G2603T) connected with linezolid resistance was also reported in 2015 (Cidral et al., 2015).

Specificity of the S. haemolyticus genome

In 2005, Takeuchi et al. (2005) sequenced the entire genome of multiresistant S. haemolyticus strain JCSC1435 consisting of 2685015 bp and three plasmids: 2300, 2366 and 8180 bp. The obtained sequence was compared with the genomes of S. aureus and S. epidermidis reference strains. A unique feature of the S. haemolyticus JCSC1435 chromosome is that it contains as many as 82 insertion sequences. Two insertion sequence groups (ISSha1 and IS1272-SH) comprised 68% of the insertion sequence elements. IS1272-SH of S. haemolyticus exhibited 85% nucleotide identity to IS1272-SA of S. aureus and 81% identity to IS1272-SE of S. epidermidis. Moreover, IS1272-SH was almost identical to the insertion sequence fragment found in type I and IV SCCmec MRSA, which indicates that it might have been transferred from S. haemolyticus to S. aureus via SCCmec cassette transfer. The presence of so many insertion sequences is presumably the reason for the frequent genome rearrangements in S. haemolyticus.

The genetic diversity of S. haemolyticus combined with the tendency to treat infections with broad-spectrum antibiotics results in the selection of multiresistant strains. SNP is also suggested to be of significant importance in the process of multiresistant strains selection (Cavanagh et al., 2014). Cavanagh et al. (2014) compared 126 genomes of S. haemolyticus strains with a reference strain of S. haemolyticus JCSC1435 and identified a mean of 10495 SNPs per genome. A significant genetic diversity was observed, amongst others, in the region of the SCCmec cassette. In 2014, a team of researchers from Norway and the UK published conclusions regarding sequencing 134 clinical strains of S. haemolyticus, isolated between 1988 and 2010 in eight countries from different geographical regions (Belgium, Germany, Japan, Norway, Spain, Switzerland, UK and USA) (Cavanagh et al., 2014). The majority of them (77%) were multiresistant, defined as resistant to at least three antibiotics. This study suggested that multiresistant strains spread clonally between countries. Multiresistant strains displace the susceptible strains in hospitals as a result of selective pressure in the environment (Holden et al., 2013; Miragaia et al., 2007; Zhang et al., 2013). An alternative hypothesis assuming an independent emergence of the multiresistant strains in different geographical regions seems to be less probable (Cavanagh et al., 2014).

Conclusion

CNS often constitute underestimated aetiological factors of human infections. One of the most important species in this group is S. haemolyticus. Despite the fact that it does not possess as many virulence factors as S. aureus and hence its potential to cause severe infections is lower, the key role in the evaluation of a threat posed by S. haemolyticus is its multiresistance. The unusual genome plasticity manifested by a large number of insertion sequences and identified SNPs might contribute to its acquisition of antibiotic resistance, and also resistance to the ‘last-chance’ antimicrobial drugs. It is suggested that S. haemolyticus might be the reservoir of resistance genes (and SCCmec cassettes) for other staphylococci (including S. aureus). Taking into consideration the great adaptability and ability to survive in the hospital environment (including on medical devices), S. haemolyticus becomes a crucial factor in HAIs caused by multiresistant staphylococci. Future studies leading to our further understanding of the mechanisms of transfer of resistance genes, including SCCmec cassettes, to other staphylococci species should become a priority, especially in view of the increasing resistance to the majority of available antimicrobial agents and increasingly difficult treatment of infections caused by multiresistant strains.

References


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