

Prevalence of *Staphylococcus aureus* and antibiotic resistant *Staphylococcus aureus* in public transport in Bratislava, Slovakia

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Abstract: There is evidence that the transmission of *Staphylococcus aureus* and methicillin-resistant *S. aureus* between the community and environmental surfaces still exists. Even the means of this transmission remain uncertain, the public transport system may serve as a potential source of different bacteria, and the contact with contaminated public surfaces may increase the risk for bacterial diseases emergence. This study aimed to investigate *S. aureus* contamination on Bratislava's public transport vehicles. Forty samples of hand-touched surfaces were collected during December 2015 and March 2017 by using surface sampling method. *S. aureus* was detected in all analysed swabs. Simultaneously, antibiotic resistance of *S. aureus* from swabs was evaluated. Of 40 samples, only 23 % did not contain *S. aureus* resistant to some of 10 analysed antibiotics. On the other hand, the severe prevalence of highly resistant *S. aureus* to penicillin, methicillin, ampicillin, and cefoxitin was confirmed. 15 % of isolates displayed resistance to at least three antimicrobial classes. The amount of *S. aureus* was not significantly influenced by the lines or by the analysed surface (grabs rails or on-board stop buttons). However, there was a statistically significant effect of year period, both between samples from December and March and between samples from the same month but different year. The study confirmed the widespread occurrence of resistant *S. aureus* in public transport vehicles in Bratislava, Slovakia.

Keywords: antibiotic resistance, Bratislava, environmental contamination, public transport, *Staphylococcus aureus*

Introduction

Public vehicles, among other public areas, are places with easy transfer of bacteria from individual to individual, and connecting people with different social, ethnic, hygienic and health conditions. The bacteria transmission may be unsettling if pathogenic and resistant bacteria occur. While some bacteria are mainly nosocomial, some are also community-acquired. These include coagulase-positive staphylococci and *Staphylococcus aureus*, which frequently colonize the nasal vestibule and the skin (ECDC, 2017) of about 10–50 % asymptomatic human. However, *S. aureus* disposes of a remarkably wide range of virulent factors leading to smooth and skeletal muscle paralysis, blood vessels damage, the formation of extensive skin lesions and finally affecting the central nervous system (Arbuthnott et al., 1990).

Moreover, pathogenic *S. aureus* is regarded as a “superbug”, due to its amazing capacity to be resistant to a wide range of antibiotics. Strains resistant to methicillin (MRSA), vancomycin (VISA/VRSA) and many other antibiotics represent an urgent global problem in community as well as in hospital-acquired infections. The mortality of *S. aureus* bacteraemia remains approximately 20–40 % despite the availability of effective anti-

microbials (Mylotte et al., 1987; ECDC, 2017). Most hospital-acquired MRSA in Europe belongs to only five clonal lineages, which have distinct geographical patterns of occurrence, whereas the background populations of methicillin-susceptible *S. aureus* (MSSA) are highly diverse, consisting of many lineages that have been widely disseminated due to leisure travel and migration flows (Gaymard et al., 2016).

In the community, *S. aureus* can be quickly transferred from carriers or infected persons via direct contact (Stepanović et al., 2008). It has also been shown that they can survive under dry conditions and can persist on different surfaces for a very long time (Bremer et al., 2004). Moreover, it is well known for its ability to stick to plastic surfaces. Teichoic acids are essential compounds present in the cell wall and are important for adhesion on plastic surfaces thanks to its interaction with other surface polymers (Reffuveille et al., 2017). In this connection, public vehicles may allow its transmission due to the high number of hand-touched surfaces and their permanent contact with transport users (Gaymard et al., 2016).

Bratislava is the capital and the biggest city in the Slovak Republic. Annually, the city public transport provides transfer for approximately 250.000 people in approximately 850 vehicles

(buses, trams, and trolleybuses). These may serve as a critical reservoir of staphylococci, due to their ability to colonize human skin and also environmental surfaces. In this connection, we decided to describe the prevalence of *S. aureus* and its antibiotic resistance profile on hand-touched surfaces in vehicles moving around the capital city of Slovakia.

Materials and methods

Sampling

In December 2015, March 2016, December 2016 and March 2017, 40 samples from 5 different public transport lines were collected by using a surface sampling method. Samples were taken concerning to have a high level of skin-to-touch surfaces, like grab rails and on-board stop buttons. The public transport lines were chosen concerning copy the routes with high passengers' frequency, such as proximity to Central bus or train station, proximity to Central Hospital and cemetery or students Hall of residences (Fig. 1).

The samples were collected from a predetermined area in vehicles (about 300 cm²) using sterile swab moistened in sterile peptone-saline solution. The samples were kept refrigerated at 4–6 °C until they were brought in the laboratory (not longer than 1 hour).

In Fig. 1, empty crosses represent proximity of the hospital, filled crosses represent proximity of cemetery and triangles represent proximity to the Hall of residence. Solid black circles represent places of sampling.

Determination of *S. aureus* and *S. aureus* resistant to antibiotics

Samples (without dilution and after their 10-fold dilution in peptone-saline solution) from each swab were plated onto Baird-Parker agar (Sigma-Aldrich, St. Louis, USA) in order to determine the total counts of *S. aureus* according to EN ISO 6888-1 (2003). Samples from each swab were also spread on Baird-Parker agar containing different antibiotics (ampicillin, ciprofloxacin, ceftioxin, erythromycin, gentamicin, chloramphenicol, tetracycline, vancomycin, penicillin, methicillin), all purchased from Sigma-Aldrich (St. Louis, USA). Antibiotic resistance was detected according to European (EUCAST, 2018) or for methicillin-resistance according to US (CLSI, 2007) resistance breakpoints. Before bacteriological counting, the plates were incubated for 48 h at 37 °C aerobically. Each experiment was run in triplicate. The suspected colonies were confirmed by tube coagulase test, by the presence of catalase by using 3 % (v/v) solution of hydrogen peroxide, by microscopy examination and by MALDI-TOF technique.

Statistical analysis

The data analysis was performed using Microsoft Excel tools 2007 (Microsoft, Redmond, Washington, USA). Data were treated by independent Student t-test and confirmed by ANOVA test with statistical significance of $p < 0.05$.

Results and discussion

In all samples, the presence of *S. aureus* was confirmed. Its counts ranged from 1.40 to

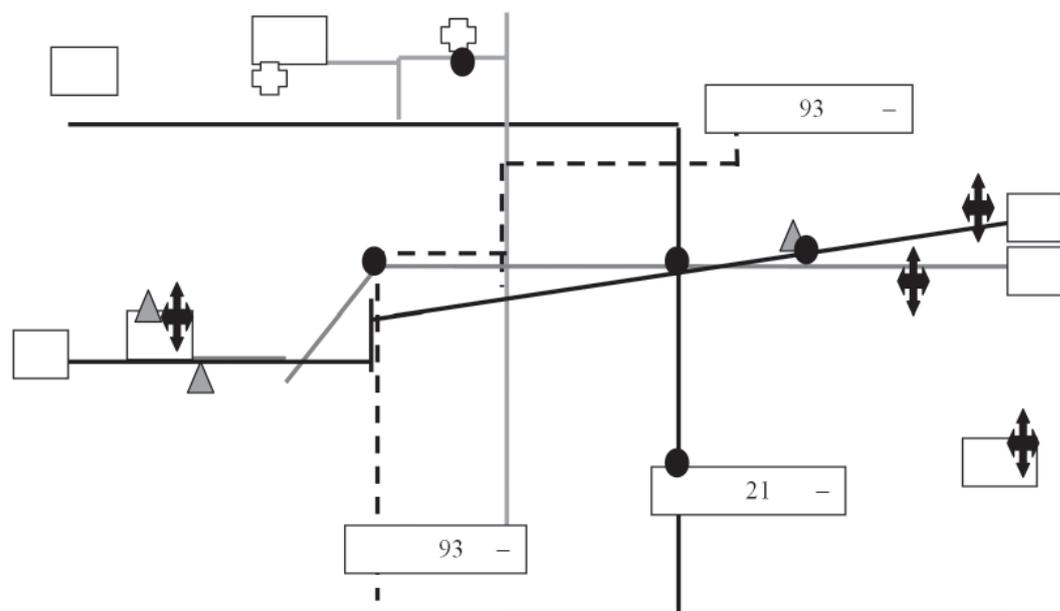


Fig. 1. Scheme of selected public transport lines in Bratislava.

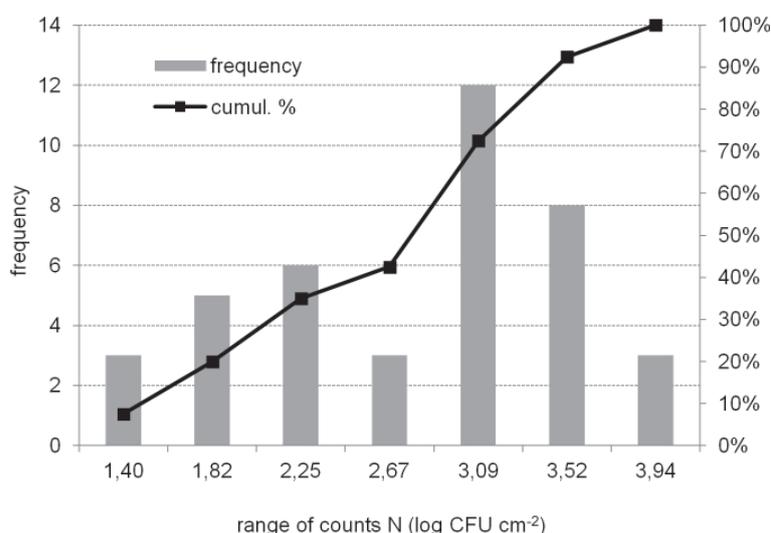


Fig. 2. The concentration of *S. aureus* contamination on hand-touched surfaces in vehicles ($n = 40$).

3.94 log CFU cm⁻², with average value of $N = 2.65 \pm 0.70$ log CFU cm⁻² (%V = 26.6 %). The distribution of staphylococcal contamination on analysed surfaces is presented in Fig. 2. Also, Sexton et al. (2007) detected MRSA in all 2959 samples from subways, buses, trains, and other public surfaces. MRSA was also isolated from all 55 vehicles analysed by Stepanović et al. (2008). However, the proportion of samples positive for the presence of MRSA per vehicle ranged from 4.5 % to 57.1 %. Similar results were also obtained by Otter and French (2009) who analysed the occurrence of *S. aureus* in buses, trains, stations, and other public places in London. They found out that 95 % of analysed surfaces were colonized by *S. aureus* and its average density was about 1.08 CFU cm⁻². On the other hand, in Lyon only 22 % of analysed surfaces in buses were positive for *S. aureus* presence (Gaymard et al., 2016) and in Porto, only 16 % of buses (mean count 6.1 CFU 100 cm⁻²) and 9 % of trains (mean count 4.0 CFU 100 cm⁻²) was contaminated by *S. aureus* (Mendes et al., 2015). These differences could be a result of specifically targeted sampling places close to hospitals in some studies. Moreover, as reported Mendes et al. (2015), the prevalence of *S. aureus* in public transport system in Porto decreased from 26 % in 2009–2010 to 9–16 % in 2015, mainly as a result of proper cleaning and disinfection practices of vehicles' surfaces. In our samples, the amount of *S. aureus* was not significantly influenced ($p < 0.05$) by the lines or by the analysed surface (grabs rails or on-board stop buttons). No significant difference between mean counts of *S. aureus* in buses and trains was also found out by Mendes et al. (2015). However, there was statistically significant ($p < 0.05$) effect of the

year period, both between samples from December and March and between samples from the same month but different year.

The occurrence of resistant *S. aureus*

The occurrence of *S. aureus* resistant to 10 different antibiotics is summarized in Figure 3, where are shown all analysed samples and samples positive for the presence of *S. aureus* resistant to selected antibiotics. Only 22.5 % of samples did not contain resistant *S. aureus* at all. 37.5 % of samples were positive for the presence of *S. aureus* resistant to only one antibiotic, mostly to a penicillin (36.8 % of them) and methicillin (31.6 % of them). These two antibiotics, together with vancomycin were the most ineffective antibiotics in general. Altogether, 50 % of samples contained *S. aureus* resistant to penicillin, and their portion in samples ranged from 67 to 99 %. There was also a high prevalence of resistant *S. aureus* to ampicillin (65–85 %) and cefoxitin (81–95 %). However, these isolates were detected only in 5 or 6 swabs, respectively.

The resistance to methicillin in analysed swabs ranged within 75 to 95 %, and 30 % of swabs were positive to the presence of methicillin-resistant *S. aureus*. These findings are in accordance with the data presented in the Antimicrobial resistance surveillance report (ECDC, 2017). While the prevalence of MRSA strains in EU countries is continuously decreasing from 18.8 % in 2012 to 16.8 % in 2015, in Slovakia a significantly increasing trend is observed. Their prevalence in 2012 was 21.7 %, and in 2015 it was 28.1 %. MRSA strains remain a public health priority not only in Slovakia but also in whole Europe, as MRSA percentages remain high (34.1 to 57.2 %) in several countries, e.g., Italy, Greece, Cyprus,

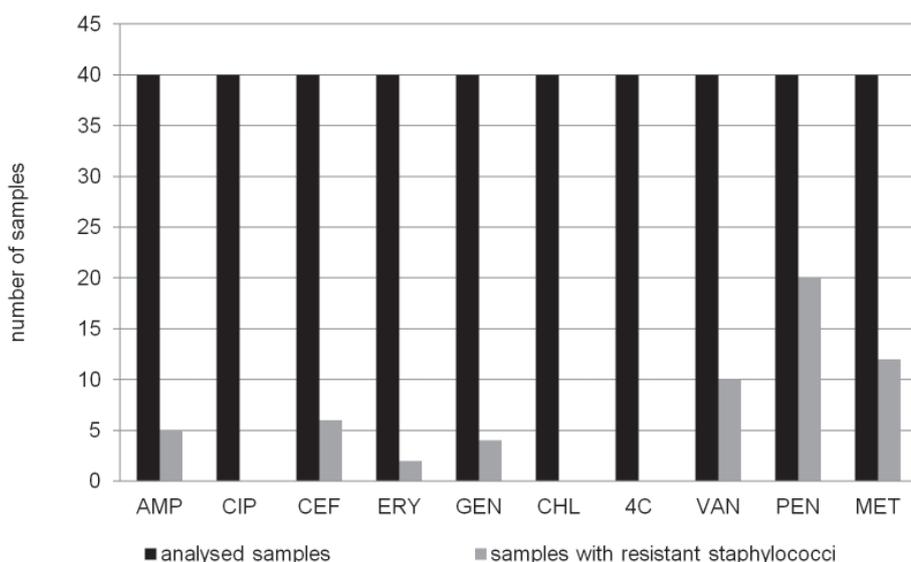


Fig. 3. The ratio of total analysed samples (n = 40) and samples with *S. aureus* resistant against selected antibiotics. AMP – ampicillin, CIP – ciprofloxacin, CEF – cefoxitin, ERY – erythromycin, GEN – gentamicin, CHL – chloramphenicol, 4C – tetracycline, VAN – vancomycin, PEN – penicillin, MET – methicillin.

Portugal, Malta, and Romania. There is also a high prevalence of MRSA strains in the United States (0.2 isolates pre 1,000 patient days) with Panton-Valentine leukocidin-positive strains as a predominant compared with the United Kingdom (0.1 per 1,000) with Panton-Valentine leukocidin-negative strains as a predominant ones (Otter et al., 2009). The increased MRSA infections reported in different countries led to the detection of unique SCCmec element, that appears more genetically mobile, and it is suggested that it has been heterologously transferred from other staphylococcal species (Lowy, 2014). MRSA isolates are often also resistant to fluoroquinolones, further limiting the treatment options available for severe infections. In this context, it was surprising, that there was no ciprofloxacin-resistant *S. aureus* in our samples.

Furthermore, there was also no *S. aureus* resistant to chloramphenicol and tetracycline. Contrary results obtained by Stepanović et al. (2005) indicating a high prevalence of coagulase-negative staphylococci resistant to tetracycline (63.1 %), followed by gentamicin (33.7 %) and erythromycin (33 %) resistant strains. From vehicles moving around Bratislava, *S. aureus* resistant to gentamicin and erythromycin were confirmed in 10 or 5 % of swabs, respectively. Stepanović et al. (2005) also found out that 46.9 % of staphylococci detected in Belgrade's public transport were multidrug-resistant. In our case, only 15 % of isolates can be considered as multidrug-resistant *S. aureus* according to criteria proposed by Magiorakos et al. (2011).

Conclusion

In conclusion, we found out that *S. aureus* was detected in all samples from public transport surfaces. Only 23 % of samples did not contain *S. aureus* resistant to some of 10 analysed antibiotics. On the other hand, the severe prevalence of highly resistant *S. aureus* to penicillin, methicillin, ampicillin, and cefoxitin was confirmed. So, public surfaces can be a significant reservoir not only for *S. aureus* but also for its resistant variants.

Further work is required to assess the prevalence of *S. aureus* also in other Slovak and European cities and also to assess the transfer of resistant bacteria from the community to environment, and vice versa. Also, the appropriate cleaning and disinfection practices are needed in order to reduce the prevalence of pathogenic bacteria in the environment together with appropriate personal hygiene practices. Last (but) not least, the well-considered and reasonable usage of antibiotics is a global necessity.

Acknowledgment

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