Etiological Structure, Drug Resistance and Biofilm Forming Capabilities of Isolates from Respiratory System from Outpatients

Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski"

Lena Ilieva, Sonya Kostadinova, Mariana Marhova, Marinela Tsankova, Ivan Iliev

Abstract

The study is focused on the etiological structure and drug resistance of bacterial strains causing upper respiratory tract infections, which are widely spread amongst children. The aim is to update the data about antimicrobial susceptibility and to establish virulence factors important for infectious process for *Staphylococcus* spp. isolates from the upper respiratory tract. A total of 711 strains were isolated from outpatients with nasal and oropharyngeal infections at Medical Diagnostic Laboratories „Sinevo-Bulgaria" LTD in Plovdiv for a two-year period 2019-2020. The results demonstrate that the etiological structure is highly related to the site of infection. *Branhamella catarrhalis* was found to dominate in the samples from the oropharynx (40%), while the majority of isolates from the nasal and ear samples were identified as *Staphylococcus aureus* - 65% and 35% respectively. *S. aureus* showed higher drug resistance compared to other isolates, reaching up to 70% to penicillins. Over 20% of the *Staphylococcus* strains were also resistant to macrolides, fluoroquinolones and aminoglycosides. Methicillin resistance was established for 39% strains. The ability of *S. aureus* to form biofilms was tested as significant virulence factor by cultivation on unmodified and modified (supplemented with 5% human plasma) tryptic Soy Broth (TSB). The analysis shows that only 16,3% *St. aureus* were capable to form stable biofilm on TSB. The addition of human plasma increases the number up to 87%. The susceptibility profile of the investigated strains in the present study confirms need always to detect resistance before antibiotic prescriptions from physicians.

Materials and Methods

Bacterial strains. The tested strains were collected from samples of outpatients at SMDL "Sinevo-Bulgaria" ltd. in Plovdiv for a two-year period from January 2019 to December 2020. The identification was conducted at three steps – standard biochemical identification, followed by confirmatory test using MERLIN MICRONAUT and 16S rRNA Sanger sequencing.

Antibiotic susceptibility testing. The antimicrobial susceptibility pattern was obtained by directions of EUCAST version 10, 2020.

Biofilm formation assay. Biofilm-forming capabilities were tested in microtiter assay with crystal violet technique as described by Agarwal & Jain (2013).

Results

By the end of 2020, a total of 2085 samples were analyzed – 366 were positive, of which 224 oropharyngeal (61%) and 142 were nasal (39%), with no co-infections. The seasonal dynamics of upper respiratory tract infections (URTIs) caused by the isolated etiological agents as well as the age structure of the patients were analyzed. The results showed that the children under 10 years of age were the most affected group with significant prevalence of the infection rate during winter period (Figures 1 and 2).

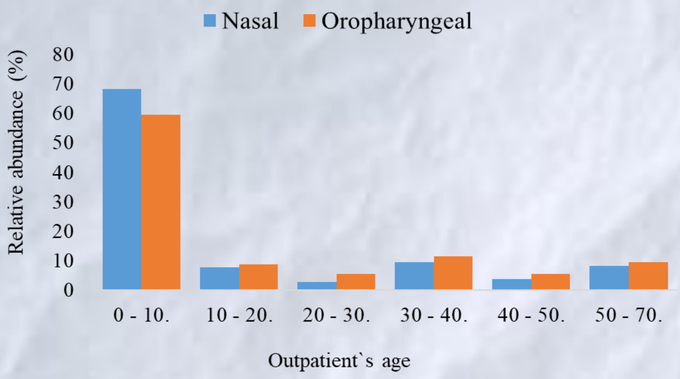

Figure 1. Age structure of outpatients with URTIs tested in SMDL „Sinevo-Bulgaria", Plovdiv.

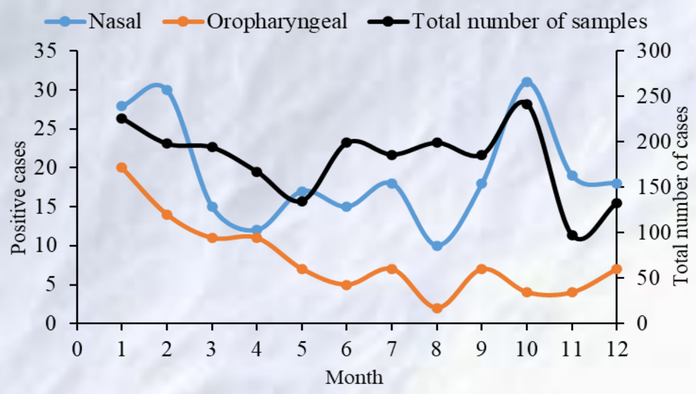

Figure 2. Seasonal dynamic of outpatients with URTIs tested in SMDL „Sinevo-Bulgaria", Plovdiv.

After three-steps identification the results showed that *Staphylococcus aureus* dominate as etiological agent, causing nasal infections (65%) while *Branhamella catarrhalis* was the most frequently identified agent in the samples form the oropharynx (40%). The results demonstrate that the etiological structure is highly related to the site of infection (Figure 3).

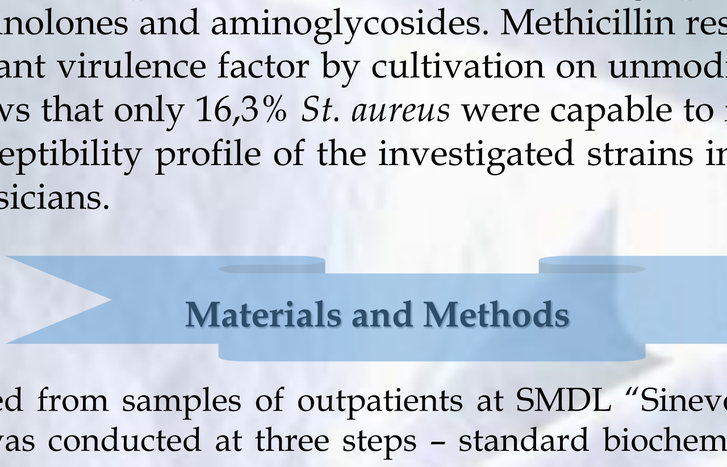

Figure 3. Etiological structure of isolated from upper respiratory tract.

The antimicrobial susceptibility was determined using the Kirby-Bauer disc diffusion method (Biemer, 1973). Strains were defined as susceptible or resistant according to EUCAST version 10, 2020.

S. aureus strains were characterized by the highest level of resistance, reaching up to 70% for penicillins (70%). High level of resistance was also detected for macrolides, fluoroquinolones and aminoglycosides (Figure 4).

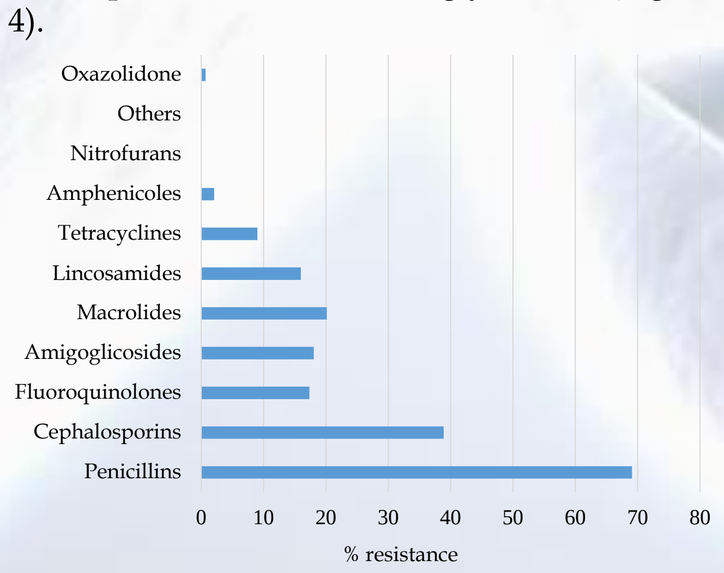

Figure 4. Antimicrobial resistance of strains isolated from outpatients with URTIs.

We used Cristal violet assay to study the biofilm-forming ability of *Staphylococcus* isolates on polystyrene plates after 24 hours cultivation on TSB medium at 37°C.

Additionally the effect of supplementation of TSB with 2% glucose and 5% NaCl (TSBGS) and TSB medium with 5% human plasma (TSBGSP) on biofilm formation was also tested. The results are presented on (Figure 5).

Total of 186 *Staphylococcus* spp. strains were able to form stable biofilm – 171 of the isolates belong to *S. aureus*, 9 isolates *S. epidermidis* and 6 strains *S. hominis*.

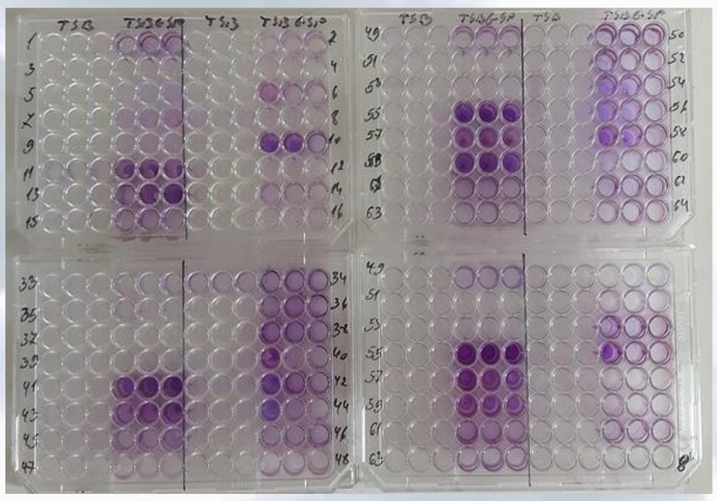

Figure 5. Biofilm-forming capacity of the tested strains with TSB, TSBGS and TSBGSP.

The performed ANOVA analysis revealed statistically significant differences between the different variants. Biofilm formation when cultivating on TSB is established for only 4. 3% of all 214 strains tested. In a variant with 5% blood plasma, biofilm formation was reported in 87% of the test strains, and there was also a significant increase in the intensity of biofilm-formation (Figure 6A). 56% of the tested strains formed a strong and stable (OD_{610} > 0.75) biofilm (Figure 6B).

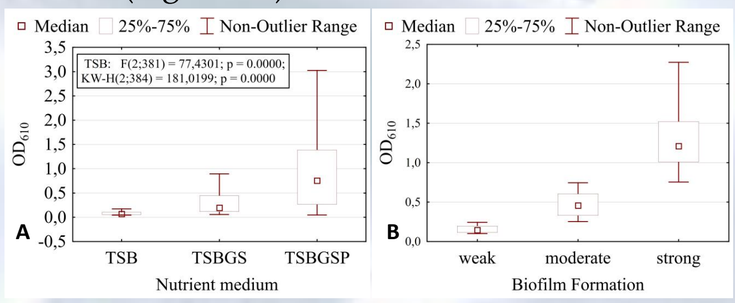

Figure 6. A) Comparison of the median value of OD_{610} in the cultivation of variously modified TSB medium. **B)** Categorization of strains based on absorption at 610nm.