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A comparison of colonization of the upper respiratory tract by Haemophilus influenzae and Staphylococcus aureus in healthy pre-school children exposed and unexposed to tobacco smoke

Porównanie kolonizacji górnych dróg oddechowych przez Haemophilus influenzae i Staphylococcus aureus u zdrowych dzieci w wieku przedszkolnym eksponowanych i nieeksponowanych na dym tytoniowy

INTRODUCTION

The ecosystem of the upper respiratory tract microflora may be a reservoir of bacterial pathogens, e.g. *Haemophilus influenzae* or *Staphylococcus aureus*, responsible for the community-acquired infections, especially in young children [11, 14]. The carriage of potentially pathogenic microorganisms within upper airways in children during the first years of life depends on various epidemiological and socioeconomic factors. There are controversial literature data concerning passive smoking as a risk factor predisposing to colonization of the upper respiratory tract by some bacteria [1, 2, 5, 7, 9].

The aim of our studies was to investigate if exposure to tobacco smoke had an effect on the rate of the upper respiratory tract colonization by *H. influenzae* and *S. aureus* in healthy pre-school children.

MATERIAL AND METHODS

During our experiments pre-school children (3–5 years old) were divided into two groups: 268 children attending a day care center (DCC group) and 76 staying at home (control group), were included. According to the parents' information, 159 (46.2%) children were exposed to tobacco smoke (parents or household members smoke in the presence of a child 2 or more cigarettes a day), whereas 185 individuals did not have contact with tobacco smoke.

Isolates of *Haemophilus influenzae* and *Staphylococcus aureus* were selected from swabs taken from throat and both nostrils (three samples from one child). The specimens were immediately

placed onto the selective media (*Haemophilus* chocolate agar and Chapman agar in the direction of *H. influenzae* and *S. aureus*, respectively) and then incubated in the appropriate atmosphere (with or without the increased CO_2 concentration) for 18–48 hrs at 35°C. The isolated microorganisms were identified on the basis of routine diagnostic methods (macroscopic, microscopic or biochemical assays) or by rapid commercial latex test – Slidex Staph-Kit (bioMérieux).

Statistical analyses included Fisher exact test and determination of relative risk (RR) was done using GraphPad InStad version 3 software.

RESULTS

It was found that the overall prevalence of *H. influenzae* in the upper respiratory tract of the assayed children was 11.3%, similarly in DCC group and in control group - 11.9% and 9.2%, respectively. The prevalence of *S. aureus* was 37.2%, also similarly in DCC group and in control group - 35.1% and 44.7%, respectively.

As presented in Table 1, in case of colonization of the upper respiratory tract by *H. influenzae*, the statistically significant difference among children exposed and unexposed to tobacco smoke was observed, both in the total population (16.3% vs. 7%) and in DCC group (17.2% vs. 7.1%). No statistically significant difference between passive smoking and nonsmoking children in the home group (12.9% vs. 6.7%), was found despite relatively high RR > 1.94, most probably due to the low number of children colonized by *H. influenzae*. There was no statistically significant difference in the prevalence of *S. aureus* within the upper airways in passive smoking children compared to those unexposed to tobacco smoke (Table 2).

| Table 1. Influence of exposure to tobacco smoke on colonization of the upper respiratory tract by |
|---|
| Haemophilus influenzae in healthy preschool children |

| Group of children | No. (%) of exposed children | No. (%) of unexposed children | <i>p</i> (RR) |
|----------------------------|-----------------------------|-------------------------------|---------------|
| DCC group $(n = 268)$ | 22 (17.2) | 10 (7.1) | 0.014 (2.41) |
| Control group ($n = 76$) | 4 (12.9) | 3 (6.7) | 0.43 (1.94) |
| Total (n = 344) | 26 (16.3) | 13 (7.0) | 0.0098 (2.33) |

 Table 2. Influence of exposure to tobacco smoke on colonization of the upper respiratory tract by

 Staphylococcus aureus in healthy preschool children

| Group of children | No. (%) of exposed children | No. (%) of unexposed children | <i>p</i> (RR) |
|------------------------|-----------------------------|-------------------------------|---------------|
| DCC group $(n = 268)$ | 43 (33.6) | 51 (36.4) | 0.7 (0.92) |
| Control group (n = 76) | 18 (58.1) | 16 (35.5) | 0.06 (1.63) |
| Total (n = 344) | 61 (38.4) | 67 (36.2) | 0.73 (1.06) |

DISCUSSION

Several studies have been undertaken to assess risk factors for the prevalence of the main respiratory pathogens, including *H. influenzae* and *S. aureus* within the upper respiratory tract [2, 5]. An important factor appears to be exposure to tobacco smoke, resulting in damage of respiratory mucous membranes, facilitating bacterial adherence to airways epithelial cells and disturbance of host defense mechanisms [14].

Our data showed that exposure to tobacco smoke may predispose to increased colonization of the upper respiratory tract by *H. influenzae* in healthy pre-school children aged 3–5 years old, especially those attending day care centers. However, data of Greenberg *et al.* [5] and Principi et al. [13] indicate that exposure to tobacco smoke did not influence the *H. influenzae* carriage in the upper respiratory tract in children aged < 5 years. According to Pereiró et al. [12], children exposed to tobacco smoke had an increased risk for diseases caused by serotype b of *H. influenzae*. It should be noted that *H. influenzae* in one of gram-negative rods comprising the commensal flora of the upper respiratory tract [8, 10]. In young children it is the most common bacterial pathogen to cause respiratory infections (e.g. acute respiratory infection, pneumonia, otitis media), or invasive infections (e.g. bacteremia, meningitis) [8, 10].

According to our data, exposure to tobacco smoke had no noticeable effect on *S. aureus* prevalence in the upper respiratory tract. However, Durmaz et al. [6] found significant differences in *S. aureus* nasal carriage in smoking and nonsmoking people but in the adult population.

Discrepancies between our observations and literature data suggest that the effect of exposure to tobacco smoke on the *H. influenzae* or *S. aureus* prevalence in the upper respiratory tract may depend on various individual or socioeconomic factors, e.g. viral or bacterial respiratory infections, previous antibiotic treatment, age, being in large group (e.g. attending day care center), geographic area [4, 6, 11, 15].

CONCLUSIONS

The obtained data suggest that in young children, especially those attending day care centers, passive smoking may predispose to colonization of the upper airways by *H. influenzae* but not by *S. aureus*.

REFERENCES

- 1. Arcavi L., Benowitz N.L.: Cigarette smoking and infection. Arch. Intern. Med., 164, 2206, 2004.
- Coen P.G., Stuart J.M., Ashby D. et al.: Is it exposure smoke or to smokers which increases the risk of meningococcal disease in teenagers? Int. J. Epidemiol., 35, 330, 2006.
- Durmaz R., Tekerekoglu M.S., Kalcioglu T. et al.: Nasal carriage of methicillin-resistant *Staphylococcus aureus* among smokers and cigarette factory workers. New Microbiol., 24, 143, 2001.
- Greenberg D., Broides A., Blancovich I. et al.: Relative importance of nasopharyngeal versus oropharyngeal sampling for isolation of *Streptococcus pneumoniae* and *Haemophilus influenzae* from healthy and sick individuals varies with age. J. Clin. Microbiol., 42, 4604, 2004.

- Greenberg D., Givon-Lavi N., Broides A. et al.: The contribution of smoking and exposure to tobacco smoke to *Streptococcus pneumoniae* and *Haemophilus influenzae* carriage in children and their mothers. Clin. Infect. Dis., 42, 897, 2006.
- Gunnarson R.K., Holm S.E., Soderstrom M.: The prevalence of potentially pathogenic bacteria in nasopharyngeal samples from individuals with a long-standing cough-clinical value of a nasopharyngeal sample. Family Practice, 17, 150, 2000.
- Iles K., Poplawski N.K., Couper R.T.L.: Passive exposure to tobacco smoke and bacterial meningitis in children. J. Pediatr. Child Health, 37, 388, 2001.
- Moxon E.R., Wilson R.: *The role of Haemophilus influenzae in the pathogenesis* of pneumonia. Rev. Infect. Dis., 13, Suppl 6, 518, 1991.
- 9. Murphy T.F.: Otitis media, bacterial colonization and smoking parent. Clin. Infect. Dis., 42, 904, 2006.
- Murphy T.F.: Respiratory infections caused by non-typeable *Haemophilus influenzae*. Curr. Opin. Infect. Dis., 16, 129, 2003.
- Peerbooms P.G.H., Engelen M.N., Stokman D.A.J. et al.: Nasopharyngeal carriage of potential bacterial pathogens related to day care attendance, with special reference to the molecular epidemiology of *Haemophilus influenzae*. J. Clin. Microbiol., 40, 2832, 2002.
- Pereiró I., Díez-Domingo J., Segarra L. et al.: Risk factors for invasive disease among children in Spain. J. Infect., 48, 320, 2004.
- Principi N., Marchisio P., Schito G.C. et al.: Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. Pediatr. Infect. Dis. J., 18, 517, 1999.
- Safdar N., Maki D.G.: The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant *Staphylococcus aureus, Enterococcus*, gram-negative bacilli, *Clostridium difficile* and *Candida*. Ann. Intern. Med., 136, 834, 2002.
- Zalacain R., Sobradillo V., Amilibia J. et al.: Predisposing factors to bacterial colonization in chronic obstructive pulmonary disease. Eur. Respir. J., 13, 343, 1999.

SUMMARY

The effect of tobacco smoke exposure on colonization of the upper respiratory tract by *Haemophilus influenzae* and *Staphylococcus aureus* was assessed in healthy pre-school children (3–5 years old). A total of 344 children, including 268 attending a day care center and 76 staying at home were assayed. For identification of the bacterial isolates, routine diagnostic methods were used. Exposure to tobacco smoke statistically significantly increased the colonization of the upper respiratory tract by *H. influenzae*, but not by *S. aureus*. The rate of *H. influenzae* colonization in exposed and unexposed children in the total population was 16.3% vs. 7.0% (p = 0.0098, RR = 2.327) and in children attending day care centers – 17.2% vs. 7.1% (p = 0.014, RR = 2.41). The obtained data suggest that in young children, especially those attending day care centers, passive smoking may predispose to colonization of the upper airways by *H. influenzae*.

Keywords: Haemophilus influenzae, Staphylococcus aureus, upper airways colonization, passive smoking, healthy pre-school children

STRESZCZENIE

W badaniach oceniano wpływ narażenia na dym tytoniowy na kolonizację górnych dróg oddechowych przez *Haemophilus influenzae* oraz *Staphylococcus aureus* u zdrowych dzieci w wieku przedszkolnym (3–5 lat). Do badań włączono 344 dzieci, w tym 268 uczęszczających do przedszkola i 76 przebywających w domu. Do identyfikacji wyizolowanych bakterii stosowano rutynowe metody diagnostyczne. Narażenie na dym tytoniowy istotnie statystycznie zwiększyło częstość kolonizacji górnych dróg oddechowych przez *H. influenzae* w porównaniu z kolonizacji u dzieci bez ekspozycji, bez wpływu na częstość kolonizacji przez *S. aureus*. Wzrost częstości kolonizacji przez *H. influenzae* stwierdzono zarówno w ogólnej populacji – z 7,0% na 16,3% (p = 0,0098, RR = 2,327), jak i u dzieci przebywających w przedszkolach – z 7,1% na 17,2% (p = 0,014, RR = 2,41). Uzyskane dane sugerują, że u małych dzieci, zwłaszcza przebywających w przedszkolach, bierne palenie może być czynnikiem predysponującym do kolonizacji górnych dróg oddechowych przez *H. influenzae*.

Słowa kluczowe: Haemophilus influenzae, Staphylococcus aureus, kolonizacja górnych dróg oddechowych, bierne palenie, zdrowe dzieci przedszkolne