SCREENING OF THROAT, CONJUNCTIVA AND FAECES CARRIER STATES AMONG NURSERY CHILDREN IN HASEKI TRAINING HOSPITAL◆

(Haseki Eğitim ve Araştırma Hastanesindeki Kreş Çocuklarında Boğaz, Konjonktiva ve Fekal Taşıyıcılık Durumlarının Araştırılması)

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Summary

In this study, 52 children who are attending at a nursery school serving only for the workers of the Haseki Education and Research Hospital are examined for their throat, conjunctiva and faeces carrier states. From throat cultures we identified 23 coagulase-negative staphylococci, 21 Staphylococcus aureus, 12 beta hemolytic streptococci, 10 Haemophilus haemolyticus, 5 Haemophilus influenzae. Neisseria meningitidis was identified from the throat culture of one case. The stool specimens were examined to screen for Salmonella and Shigella spp. In only one case, ampicillin-sulbactam resistant Salmonella spp. was detected. From the conjunctiva cultures we identified coagulase negative staphylococci and S. aureus.

Some bacteria can cause severe outbreaks in public places. With this study we have screened the carrier states of the nursery children and determined two bacteria -N. meningitidis and Salmonella spp.- which may cause outbreaks in suitable conditions.

Key words: *Nursery school, carrier state.*

Özet

Bu çalışmada sadece Haseki Eğitim ve Araştırma Hastanesi çalışanlarının çocuklarının bakıldığı gündüz bakımevine devam eden 52 çocuğun boğaz, konjonktiva ve fekal taşıyıcılık durumları araştırıldı. Boğaz kültürlerinden 23 koagulaz negatif stafilokok, 21 *S. aureus*, 12 beta hemolitik streptokok, 10 *Haemophilus haemolyticus*, 5 *Haemophilus influenzae* identifiye edildi. Bir olgunun boğaz kültüründen *Neisseria meningitidis* identifiye edildi. Gaita örnekleri *Salmonella* ve *Shigella spp.* yönünden araştırıldı. Sadece bir olguda ampisilin-sulbaktama dirençli *Salmonella spp.* saptandı. Konjonktiva kültürlerinden koagulaz negatif stafilokok ve *S. aureus* identifiye edildi.

Bazı bakteriler toplu yaşanan yerlerde ciddi salgınlara neden olabilir. Bu çalışma ile kreşteki çocukların taşıyıcılık durumları araştırıldı ve uygun koşullar oluştuğunda salgına neden olabilecek iki bakteri- *N. meningitidis* ve *Salmonella spp.*- tespit edildi.

Anahtar kelimeler: Gündüz bakımevi, taşıyıcılık durumu

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INTRODUCTION

In public places, infections by pathogen bacteria may cause serious outbreaks. In such places, when the rate of the microbial flora harboured by the hosts exceeds a certain percent, the probability of outbreaks caused by these bacteria increases.

A major question regarding child day care is whether the pattern or frequency of infectious diseases in day care centers differs significantly from that among children cared for at home. Outbreaks of several important groups of infectious diseases, including bacterial, viral, and parasitic diarrhea, hepatitis A, bacterial meningitis, and vaccine-preventable diseases such as measles and chicken pox may occur among nursery children [1].

The multiple opportunities for child-to-child contact in groups of diapered children might result in direct transmission of enteric infections, and the frequency of patogen introduction may increase by larger numbers of children in groups. Thus, it might not be possible to reduce rates in larger child groups to the level in smaller groups [1].

The mostly important bacteria in pharyngeal infections are *S. pyogenes* and *C. diphtheriae*.

Carriage of *N. meningitidis* in the nasopharynx in otherwise healthy humans has been recognised first since 1896 [2]. The most important step in pathogenesis of *N. meningitidis* infection is nasopharyngeal colonisation of the bacteria [3].

The peak incidence of reported isolates of *Salmonella spp.* in the United States among children is less than 1 year of age. Most salmonella infections acquired in a family setting are thought to result from the consumption of foods that were contaminated outside the home and then were improperly prepared or stored [4]. However, at nursery schools and hospitals person-to-person spread is also usual.

Haemophilus spp. other than *H. influenzae* are rare causes of human disease. *H. haemolyticus* rarely, if ever, causes clinical disease, whereas *H. parahaemolyticus* is an uncommon pathogen. *H. influenzae* is among the bacteria normally found in the pharynx and, to a lesser extent, it also colonises the mucosae of the conjunctiva [2].

In a healthy person there are a few bacteria on conjunctiva. The most frequent flora members are coagulase-negative staphylococci and lactobacilli. *S. aureus, Haemophilus* spp., *Moraxella catarrhalis,* enteric bacteria and streptococci are less frequent members [5].

Nursery schools are at risk for air-borne and food borne disaeases and as well as for oral-fecal transmitted disaeases proportionally to their relationship with children who have no proper toilet training. Therefore nursery schools are places where care must be given to hygienic conditions, like the observation of workers and also attenders.

METHODS

In this study, 52 children attending at a nursery school serving only for the workers of the Haseki Education and Research Hospital are screened for their throat, conjunctiva and faeces cultures to determine their flora and carrier states.

The throat cultures of the children were inoculated sheep blood agar obtained commercially and chocolate agar prepared in our laboratory. The genus and species names of the grown bacteria were identified by conventional methods and API. Bacitracin susceptibility test and PYR (L-pyrrolidonyl-α-naphthylamide) hydrolysis test were performed for β-hemolytic, catalase negative, gram positive cocci. CAMP (Christie, Atkins, and Munch-Petersen) test was also performed for all the β-hemolytic streptococci. Negative test proved none of them were group B streptococci. Negative esculin and PYR hydrolysis tests proved none of the β-hemolytic streptococci were enterococci. Antibiotic susceptibilities for *Haemophilus spp.* were examined by Kirby-Bauer disk diffusion method on chocolate agar medium and API ATB NH (Bio Merieux, France).

Conjunctiva cultures were inoculated sheep blood agar and thioglycollate broth.

Stool cultures were directly inoculated onto Endo agar medium and after enrichment for 6-8 hours in selenite F broth, to SS agar medium,all of which were prepared in our laboratory. They were

investigated for salmonella and shigella. Lactose negative, producing H_2S, motile, oxidase, urease and indole negative, methyl red positive colonies were detected for salmonella serologically with Difco antisera.

RESULTS

The children were cared for in 3 different rooms of the nursery school according to their age groups. There were 2 infants aged 0 to 12 months (3.8%), 22 toddlers aged 13 to 36 months (42.3%), and 28 children aged 3 to 6 years (53.8%).

Table 1. Distribution of child age groups

	n	%
< 1 years old	2	3,85
1-3 years old	22	42,30
> 3 years old	28	53,85

The distribution of grown bacteria is shown in Table 2.

Table 2. The distribution of grown bacteria

	n	%
Coagulase-negative staphylococci	24	46,2
S. aureus	24	46,2
Alpha hemolytic streptococci	39	75
Neisseria spp.	26	50
Beta hemolytic streptococci	15	28,8
H. haemolyticus	11	21,2
H. influenzae	6	11,5
H. parahaemolyticus	3	5,8
Pseudomonas spp.	5	9,6
Diphtheroids	4	7,7
N. meningitidis	1	1,9

All of these β-hemolytic streptococci had bacitracin resistance and negative PYR tests; none of them being identified as group A β-hemolytic streptococci.

All of the α-hemolytic streptococci were optochin resistant so none of them were identified as pneumococci.

N. meningitidis grew on chocolate agar medium taken from the throat culture of a 5 years old, asymptomatic case who has been attending the nursery school for 1 year.

Among the grown bacteria, β-hemolytic streptococci and *Neisseria spp.* were the most isolated ones.

The distribution of the 20 *Haemophilus* spp. (11 *H. haemolyticus*, 6 *H. influenzae*, 3 *H. parahaemolyticus)* grown from throat cultures were as follows;

All of the *Haemophilus spp.* were susceptible to amoxicillin-clavulanate and netilmicin. None of them were susceptible to penicillin.

Table 3. Antibiotic susceptibility rates for Haemophilus spp.

	Susceptible	İntermediate	Resistant
P	0	6	12
TE	7	5	6
E	8	8	2
C	13	2	3
AMC	18		
NET	18		
CTX	15		3
CIP	16		2
RD	14	3	1

P: Penicillin, TE: Teicoplanin, E: Erythromycin, C: Chloramphenicol, AMC: Amoxicillin-clavulanate, NET: Netilmicin, CTX: Cefotaxime, CIP: Ciprofloxacin, RD: Rifampicin

27 *coagulase-negative staphylococci* and 16 *S. aureus* were identified from conjunctiva cultures.

Methicillin resistance was screened for 16 isolated *S. aureus*. There were 9 methicillin sensitive

Table 4. The isolates from conjunctiva cultures

	n	%
Coagulase-negative staphylococci	27	51,9
S. aureus	16	30,7
No growth	9	17,3

S. aureus (56%) and 7 methicillin resistant S. aureus (44%).

In only one case *Salmonella* spp. was isolated from stool sample. It was found to be resistant to ampicillin-sulbactam in vitro, on Mueller-Hinton agar medium by Kirby-Bauer disk diffusion method. This case was asymptomatic, 2.5 years old and attending at the nursery school for one year.

CONCLUSION

The normal flora of the human body are influenced by environmental factors such as diet, sanitary conditions, air pollution, and hygienic habits [2].

Throat is among the body parts from which culture samples are taken most frequently. *Streptococcus viridans,* beta-hemolytic streptococci other than group A, coagulase negative staphylococci, peptostreptococci, *Neisseria* spp., *Haemophilus* spp., and diphtheroids form the throat flora. Group A beta hemolytic streptococcus (S. pyogenes) which is the cause of throat infections may also participate in the throat flora. Pharyngeal carriage rates among normal school-children vary with the geographic location and season of the year. Group C and G β-hemolytic streptococci may also cause pharyngitis, but laboratories do not screen for and report these isolates because of the lack of proven rheumatic fever sequelae, as well as only rare reports of associated post-streptococcal glomerulonephritis [2].

Group A beta-hemolytic streptococci cause infections mostly among children aged 5-15 years. Various studies were performed in our country showed beta-hemolytic streptococcus rate on throat cultures of primary school children ranged between 7.47%-34.2% and among these bacteria 4.7%-29.1% was group A beta-hemolytic streptococci [6].

In our study including children aged under 6 years old, the rate of beta-hemolytic streptococcus was 28.8%. We observed neither group A nor group B β hemolytic streptococci in throat cultures of 52 children at the nursery school. There were no presence of pneumucocci and enterococci either.

In the population the carrier rate of nasopharyngeal *N. meningitidis* is usually 2-10 % (5).

The human body is very resistant to meningococci to cause an infection. However, meningitis caused by meningococci is more often seen if the carrier rate exceeds 20%. The rate reaches 80% during the outbreaks [5,7].

Many reports from the western countries stated that meningococcal infections make peak in childhood, especially at preschool age [8,9]. Elevli et al. had reported a study about 106 patients with the diagnosis of meningococcal infection in a pediatrics clinic of a university hospital in our country. They found out 14% of the cases were infants and 70% were under 6 years old [10].

In our study, we determined only one child with the carriage of *N. meningitidis* in his throat. This case was 5 years old and attending at the nursery school for one year. We got throat cultures from all the family members of this case and did not screen for any *N. meningitidis*. We determined the meningococcal carriage rate as 1.92% in the nursery. This rate is below the general population rate which is 2-10%.

At nursery schools and hospitals the environment should be kept clean and dry, and disinfection and sterilisation procedures should be performed perfectly in order to prevent pseudomonas colonisation. [2] There were 5 *Pseudomonas spp.* in 52 throat cultures. This makes a rate of 9.6% which is a bit higher than the rate of Pseudomonas (0-6.6%) in throat flora of general population [5].

We expected to isolate Gram negative enteric bacilli in some of the throat cultures of children at the nursery but did not isolate any of them.

H. influenzae may be situated in normal throat flora. It is reported that rate of *H. influenzae* in throat flora may reach 80%. Nasopharyngeal colonisation by *H. influenzae* in the first year of life is associated with an increased risk of recurrent otitis media compared with children who remain free of colonisation. [2] In our country *H. influenzae* infections are fewly determined. The most important reason of this is the fact that *H. influenzae* hardly grows on routinely used media. This bacteria is responsible of various invasive and noninvasive infections such as meningitis, epiglottitis, cellulitis and upper respiratory tract infections. The Haemophilus species isolated from throat cultures of children in our study, included *H. influenzae, H. haemolyticus* and *H. parahaemolyticus*. The prevalence of all Haemophilus species was 38.44% (*H. haemolyticus* 21.15%, *H. influenzae* 11.53%, *H. parahaemolyticus* 5.76%). In a study of Vahaboglu et al., the prevalence of *H. influenzae* in patients aged ≤5 years is 76.9% [11]. Akcakaya et al. studied the nasopharengeal cultures of children in a nursery school of a university hospital in our country. The prevalence of *H. influenzae* among 168 children was 61.9% [12].

These rates are much higher than the rates determined in our study. The *H. influenzae* prevalence in the above mentioned study reached the maximum level at 3 years of age (54 of total 104) and the minimum level (20 of total 104) at 5 years of age [12]. All of the cases colonised with *H. influenzae*, in our study, were aged 4 to 6 years and they were not in contact with the 0 to 1 year old and 1 to 3 year old groups.

Since 1974, there have been several reports about the increasing resistance of *H. influenzae* to antibiotics such as ampicillin and amoxicillin [13]. The first failure of ampicillin therapy in *H. influenzae* meningitis was a case reported in 1968. The first strain resistant to ampicillin was isolated from the cerebrospinal fluid of a child and reported in 1974. The first strain resistant to chloramphenicol was reported in 1972 [14]. All *Haemophilus spp.* grown on the throat cultures in our study were resistant to penicillin and susceptible to amoxicillin-clavulanate and netilmicin.

Factors which play role in transmitting salmonella and shigella are; the faeces of ill people and healthy carriers, contaminated food and water, contaminated hands and insects. Carriers play an important role in transmitting these bacteria since they are not under control. We detected *Salmonella spp.* on the stool culture of only one case. The bacteria was resistant to ampicillin-sulbactam. This case was 2.5 years old, attending at the nursery school for one year. The stool cultures of this child's all family members were examined and no *Salmonella spp.* were detected. There were no *Shigella spp.* on stool cultures of the 52 children.

Organisms mostly responsible for bacterial conjunctivitis in adults are *S. pneumoniae, S. aureus, S. epidermidis*, and in children are *H. influenzae, S. pneumoniae*, and *S. aureus* respectively.

Among 52 conjunctiva cultures 43 had growth. 27 of them were coagulase-negative staphylococci, 16 were *S. aureus*. 7 of the total *S. aureus* were methicillin resistant *S. aureus* (MRSA). There were no MRSA in the throat cultures of the children who had MRSA in their conjunctiva cultures. 2 of the total 9 children who had methicillin sensitive *S. aureus* (MSSA) in their conjunctiva cultures had MSSA also in their throat cultures. 11 of the 27 children who had coagulase-negative staphylococci (CNS) in their conjunctiva had CNS also in their throats.

Nursery schools are places where some microorganisms can easily cause outbreaks. In a nursery school, like the one in our study, serving for children of the staff of a hospital, this risk increases. Hospital staff may have many microorganisms on their hands because of contact with patients. In hospitals the personnel who are responsible for patient care have more and various kinds of bacteria on their hands. The rate of pathogen microorganisms on hands of normal people is 6%, whereas this rate increases to 68% on hands of hospital staff who do the patient care [15]. Handwashing is very important to reduce the microorganism number on hands of the hospital staff and their children and the personnel who care for the children in nursery school. Conditions of the nursery schools must be improved and vaccination programmes should be

conducted. Same regulations should be done also for the working personnel. Screenings of carrier states sometimes should be enlarged including all the family members.

KAYNAKLAR

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