Risk Analysis and Effective Factors of Pathogens on the Hands of Kindergarten Students in Istanbul - Turkey [1]

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Summary

Persons are the primary performers for all food security systems and public health procedures, but humans do not have a standard configuration. The personal variables like age, gender, economic positions, psychological conditions, educations given by the family, life style and environment are the factors that determine main personal differences of humans. This situation can cause implementation differences for standard procedures as food security performances, educations related with food hygiene. Especially individual hygiene procedures must be taught to students are being educated in preschool programs very effectively and in entertaining ways. In this study, we aim to determine how the personal variables (age, gender, social and economic positions, hand washing habits etc) effect the microbiological load of the students' hands according to the chosen microbiological parameters statsitically (plate count, total coliform group bacteria, *Escherichia coli, Staphylococcus aureus, Salmonella* spp.) who are registered preschool education (kindergarten) programs of different schools in Istanbul. For this purpose, 10 preschool education programs (kindergartens) were visited during 3 months (visits were monthly). From each school 20 students were sampled. The samples were analyzed for the chosen microbiological parameters and the correlations were determined among the microbiological parameters and the individual variables of the students.

Keywords: Foodborne pathogens, Kindergartens, Preschool education programs, Hands, Risk analysis

İstanbul'daki Anaokulu Öğrencilerinin Ellerindeki Gıda Kaynaklı Patojenlerin Etkileyici Faktörleri ve Risk Analizleri

Özet

Tüm gıda güvenlik programları ve halk sağlığı prosedürlerinde birincil uygulayıcılar insanlardır. Ancak insanların tümü standart bir yapı sergilememektedir. Sosyal bir varlık olan insanın yapısındaki ana farklılıkların oluşmasında yaş, cinsiyet, sosyo-ekonomik pozisyonlar, psikolojik durumlar, aile içi eğitimler ve bakış açıları, çevresel etkenler gibi bireylere özgü değişkenler etkin bir rol oynamaktadır. Bu durum da, hem standart bir bilginin veya eğitimin verilmesindeki kişisel farklılıklara hem de standart bilgiyi ve eğitimin alınmasını takiben her bir bireyin alınan bilgileri hayata geçirmesinde kişisel farklılıklara neden olabilmektedir. Özellikle gelişim çağını yaşayan ve okul öncesi eğitim alan çocuklara kişisel hijyen prosedürleri çok bilinçli ve dengeli bir biçimde öğretilmeli, uygulattırılmalı ve söz konusu prosedürler okul öncesi eğitim alan çocuklara eğlenceli bir alışkanlık haline getirilmelidir. Bu araştırma, İstanbul bölgesindeki okul öncesi eğitim alan (anaokuluna devam eden) öğrencilerin bireysel değişkenlerinin (yaş, cinsiyet, sosyo - ekonomik durum, el yıkama alışkanlığı vb.) seçilen başlıca gıda kaynaklı patojen bakteri parametreleri açısından (toplam mezofilik aerobik bakteri, toplam koliform grubu bakteri, *Escherichia coli, Staphylococcus aureus, Salmonella* spp.) ellerindeki mikrobiyolojik yükü nasıl etkilediğinin istatistik analizlerle belirlenmesi amaçlamaktadır. Bu amaçla İstanbul bölgesinde bulunan 10 adet anaokulu 3 ay boyunca her ay olmak üzere ziyaret edilmiş ve her bir okuldan 20 adet öğrenci örneklemesi yapılmıştır. Alınan örnekler seçilen mikrobiyolojik parametreler açısından analiz edilmiş ve analiz sonuçları ile öğrencilerin kişisel değişkenleri arasındaki korelasyon ilişkileri belirlenmiştir.

Anahtar sözcükler: Gıda kaynaklı patojenler, Anaokulları, Okul öncesi eğitim programları, El, Risk analizleri

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INTRODUCTION

Foodborne pathogens are important risk factors for public health all over the world because of their widespread existence features [1]. In the United States of America, 76 million food poisoning cases are reported every year and 50.000 cases of those cases are reported to be fatal [2]. Mass consumption points as schools, hotels, hospitals, business centers have important roles on contamination pathways and food related surfaces, equipments, staff and even final consumers are evaluated as critical control points for food safety chain [3]. Inadequate hygiene conditions and/or pathogens contaminated hands can be serious potential risk factors for public health. Systematic hygiene implementations and continuous hygiene education programs are important to decrease the incidence of contamination of foodborne pathogens. According to the laws related to the new hygiene regulations of European Community (EU), it is a compulsory process for all kinds of food plants to apply food security systems at the all steps of the production and selling practices since 2005 [4,5]. Mass consumption points as schools, hotels, hospitals, business centers have important roles on contamination ways and food related surfaces, equipments, staff and even final consumers are evaluated as critical control points for food safety chain [3].

In developed countries, one of the most important preventive factors for the public health is to provide healthy and qualified foods for balanced diets to the consumers while eliminating the possible pathogens and producing all kinds of foods under sufficient hygienic conditions. Continuously increasing population of world, pollution of the natural sources, industry, lack of education due to economic reasons complicates to provide hygienic and safe food products and enlarges nutrition problems and cause to increase the cases suffering from foodborne illnesses and toxications all over the world. Based on these reasons, it is very important to determine the contamination ways of the foodborne pathogens, and the risk factors especially in mass consumption points [6].

In this study it was purposed to determine how individual variables (age, gender, handwashing frequency, economic status etc.) of the preschool (kindergarten) students of different schools located in Istanbul effect the microbiological loads of their hands according to the chosen microbiological parameters and the results were interpreted in the aspect of formed statistical analysis.

MATERIAL and METHODS

School Visit Programs

To determine how the individual variables of the students may affect the microbiological hand loads of the students, total of 10 schools were visited during 3 months. The schools were separated into two groups according to their economic income levels (the schools which were located in the districts that had lower economic income levels relatively and the schools which were located in the districts that had higher economic income levels relatively). From each group of district, 5 schools were investigated. Twenty students (10 male and 10 female students) from each school were chosen. At every visit, same students were sampled to verify standard deviations and correlations among the variables and the microbiological parameters. Every school were visited during 3 months (one visit/month to each school) to collect sufficient hand swab samples for the statistical analysis.

Student Samplings

Hand swab samples of 20 students (10 male, 10 female) for each school were collected. Entire palm including fingers for each student was sampled by using double swab technique. All the hand swab samples were transported to the laboratory and analyzed in the same day of sampling for the chosen microbiological parameters. Same students were sampled in each school at every visit during the visiting period of the study to get clear statistic results. The rate of different student sampling because of the reasons such as changing schools, absence of the students because of illnesses and/or from individual excuses was less than 1% of total sampling process. *Table 1* shows the analyzed microbiological parameters and the individual variables of the students.

Microbiological Analysis

All the microbiological analysis were performed according to the standards of FDA/BAM (Food and Drug Administration/Bacteriological Analytical Manual) [7] and

Table 1. *Analyzed microbiological parameters and the recorded individual variables of the students*
Tablo 1. *Analiz edilen mikrobiyolojik parametreler ve değerlendirilen bireysel değişkenler*

Microbiological Parameters Analyzed	Variables of the Students Recorded
Total mesophilic aerobic bacteria count	Age
Total coliform group bacteria count	Gender
Escherichia coli	Diaper usage
Staphylococcus aureus	Hand washing frequency (number of handwashings/day)
Salmonella spp.	Economic status of the schools (according to the locations of the schools in low or high economic income regions)

double swab technique was used. All the swab samples were transported to the laboratories of Istanbul University, School of Veterinary Medicine, Department of Food Hygiene and Technology and the analysis were performed in the same day of the sample transportation for each visit program. During transportation period, tubes included Stuart Transport Medium (Oxoid, CM 0111) was used for each swab. Different swabs were used for each micro-biological parameter.

Total Mesophilic Aerobic Bacteria Count: Swabs were streaked onto Petri dishes containing Plate Count Agar. Petri dishes were incubated 37ºC for 48 h and typical colonies were counted at the end of the incubation period [7].

Coliform Group Bacteria Count: Swabs were streaked onto Petri dishes containing VRBA. After inoculation an additional VRBA layer was poured to each dish. Petri dishes were incubated 37ºC for 24 h and typical colonies were counted at the end of the incubation period [7].

Escherichia coli Count: Swabs were streaked onto Petri dishes containing TBX (Tryptone Bile X-glucuronide). Petri dishes were incubated at 44ºC for 24 h and typical colonies were counted at the end of the incubation period. Approximately 98% of *E. coli* serotypes include β - D glucuronidase enzyme. Mmentioned enzyme is found extremely rare in other pathogens and breakdowns MUG (4-Methylumbilliferyl-β-D glucuronid) substrate and the brokendown products form fluorescent radiation under UV light [8]. Therefore, an additional chromogenic medium including MUG (*E. coli* Broth with MUG) was used for verifying.

Staphylococcus aureus Count: Swabs were streaked onto Petri dishes containing BPA (Baird - Parker Agar). Petri dishes were incubated at 37ºC for 24 h. At the end of the incubation period, suspected colonies were passeged to DNAse Agarand incubated at 37ºC for 24 h. Coagulase test was performed to the suspected colonies and coagulase positive colonies were evaluated as *S. aureus* [7].

Salmonella spp. Count: Swabs firstly were pre - enriched in BPW (Buffered Peptone Water). After an incubation period at 37ºC for 24 h, isolates then enriched in Rappaport Vassiliadis Soy Broth which is a selective medium for *Salmonella* spp. Again enriched isolates were incubated at 37ºC for 24 h, then enriched cultures were streaked onto Brilliant Green Phenol Red Lactose Sucrose Agar, which is a solid specific media. Besides, XLD (Xylose Lysine Deoxycholate Agar) agar was also used for verification of detecting suspected colonies. Both XLD and Brilliant Green Phenol Red Lactose Sucrose Agar included Petri dishes were inoculated for 24 h at 37ºC and chemical tests were used to identify the suspected colonies. At this stage TSIA (Triple Sugar Iron Agar), urea broth and SIM (Semi Indol Motility) agar were used and identification procedure was completed [7].

Statistical Analysis

Pearson chi - square analysis method was used for determining the correlations among the variables. t test was used for examining the differences among the groups (gropus were detected due to the types of the schools). Correlations among individual variables and chosen micro-biological parameters were analysed in this study. Besides, significant differences between sub-groups and chosen microbiological parameters were exposed statistically [9,10]. Grouping of the variables and microbiological values were arranged according to binary system (as 0 and 1) for each variable and microbiological result. For pathogens, growing colonies were evaluated as "1" and empty petri dishes were evaluated as "0" for the chi - square tests.

RESULTS

In this study, 10 schools (kindergartens) were visited during 3 months (April - June) and from each school, the microbiological loads of 20 students' hands (10 male students, 10 female students) were analyzed for the chosen microbiological parameters. Besides, to determine the

Table 2. Student questionnaire and the detailed data bases of the related questions
Tablo 2. Öğrencilere sorulan sorular ve her sorular ve veri detayları

Individual Variables of the Students Investigated (n=200)	Sub-diffraction Topics and Values of the Related Individual Variables			
Age	3 and 3+ years old		5 and 5+ years old	
	100		100	
Gender	Male	Female	Male	Female
	50	50	50	50
Diaper using students	Male	Female	Male	Female
	22	18	4	3
Hand washing frequency (number of handwashingsday)	1-4 times	5 and over 5 times	1- 4 times	5 and over 5 times
	72	28	89	11

*Hand washing frequency variable was not depended on gender parameter, due to this reason, mentioned variable was based on the daily number of hand washing procedure

factors that affect microbiological pollution and to determine correlations between microbiological pollution and individual parameters 4 questions were asked to students which thought to be effective to microbiological pollution and total contamination. *Table 2* shows the questionnaire of the students. *Table 3* shows the correlations between individual variables and the chosen analyzed microbiological parameters of the students and *Table 4*

Table 3. Correlations between individual variables and the chosen analyzed microbiological parameters of the students
Tablo 3. Analiz edilen mikrobiyolojik parametreler ve öğrencilerin bireysel değişkenleri arasındaki ikili korelasyon ilişkileri

Variables	Microbiological Parameters	Std. Deviation	Min/Max Colony Numbers (cfu/hand palm)	95% Confidence Interval of Lower Difference	95% Confidence Interval of Upper Difference	*Chi - Square* Values	Significance
Economic Status of the Schools (acc. to the locations of the schools in low or high economic income regions)	TAB	.55123	5 - Diffuse	.47985	.98682	47.002	**
	TCB	.50022	0 - 11	.20674	.85992	35.382	**
	E. coli	.33786	0 - 6	.07526	.60355	21.178	**
	S. aureus	.38193	0 - 3	.33180	.88082	13.744	*
	Salmonella spp.	.08938	0 - 1	.13180	.66820	13.787	*
Age	TAB	.0110	5 - Diffuse	-.73241	-.1676	9.187	NS
	TCB	.14049	0 - 11	-.69373	-.1063	14.052	NS
	E. coli	.13955	0 - 6	-.65611	-.1478	6.126	NS
	S. aureus	.09597	0 - 3	-.56862	-.0314	5.743	NS
	Salmonella spp.	.12566	0 - 1	-.40065	.20065	12.913	NS
Gender	TAB	.11585	5 - Diffuse	-.3743	.27427	12.834	**
	TCB	.16018	0 - 11	-.2115	.41159	10.618	**
	E. coli	.15390	0 - 6	-.5854	-.01464	5.150	*
	S. aureus	.14049	0 - 3	-.4116	-.4115	11.585	*
	Salmonella spp.	.15390	0 - 1	-.4007	-.3992	8.730	NS
Diaper Usage	TAB	.14779	5 - Diffuse	.11464	.68536	23.057	NS
	TCB	.13908	0 - 11	.21733	.78267	16.085	**
	E. coli	.14510	0 - 6	-.0956	1.042	24.662	NS
	S. aureus	.12460	0 - 3	-.0557	1.592	31.424	NS
	Salmonella spp.	.13179	0 - 1	-.2345	.000	7.367	NS
Hand washing Frequency	TAB	.14779	5 - Diffuse	-.0334	.1000	145.581	**
	TCB	.11819	0 - 11	-.1796	.0796	221.089	**
	E. coli	.15936	0 - 6	-.1380	.0380	94.637	**
	S. aureus	.12599	0 - 3	-.0824	.0824	81.804	**
	Salmonella spp.	.13093	0 - 1	-.0881	.1881	18.470	**

* P<0.05, ** P<0.01
TAB: Total mesophilic aerobic bacteria count, **TCB:** Total coliform group bacteria count, **NS:** Not significant, **cfu:** Colony forming unit

Table 4. Comparision of the school groups due to economic status (the schools located in the regions that had relatively low economic income versus the the schools located in the regions that had relatively high economic income)
Tablo 4. Okulların ekonomik statülerine göre (görece düşük ekonomik gelir seviyesine sahip bölgelerde bulunan okullar ile görece yüksek ekonomik gelir seviyesine sahip bölgelerde bulunan okullar olmak üzere) karşılaştırılması

Parameter	F	Mean Difference	Std. Er. Difference	95% Confidence Interval of Lower Difference	95% Confidence Interval of Upper Difference	t	df	Sig. (2-tailed)
TAB	37.471	.2610	.04286	.17684	.34525	6.091	496	.000**
TCB	47.828	.1767	.04289	.09243	.26099	4.120	496	.000**
E. coli	73.935	.1084	.02679	.05577	.16110	4.048	496	.000**
S. aureus	51.373	.0723	.02112	.03076	.11382	3.423	496	.001*
Salmonella spp.	25.750	.0241	.00974	.00492	.04328	2.475	496	.014*

* P<0.05, ** P<0.01
TAB: Total mesophilic aerobic bacteria count, **TCB:** Total coliform group bacteria count

shows the comparision of the school groups due to economic status (the schools located in the regions that had relatively low economic income versus the the schools located in the regions that had relatively high economic income).

DISCUSSION

In this study, it was aimed to determine how the individual parameters (age, gender, economical status, etc.) of the preschool students were interacted with the microbiological load of the students' hand and the role of mentioned parameters and habits on total contamination ways at schools of Istanbul by using statistical analysis. Multiple and binary correlations of the parameters were exposed and the effect of the parameters on contamination pathways on schools were investigated on the basis of selected groups.

Correlations of the chosen microbiological parameters due to economic status of the schools were analyzed and statsitical differences were in favor of the schools located in the regions which had higher economical income levels relatively (the students of the schools located in higher economical income level regions were cleaner than the students of the schools located in lower economic income level regions due to the chosen microbiological parameters). It is thought that more effective hand hygiene education are both given and performed to the students in mentioned schools by their teachers and/or families. Besides, usage of more effective and high quality soaps/disinfectants may be one of the reasons of our results. Period and frequency of handwashing is also very important for eliminating the pathogens and it was observed that the students of the schools located in higher income level regions were spending approximately double time period for each handwashing process than the students of the schools located in lower economic income level regions (data not shown). Medical literature indicates that it is very important to use effective chemicals (the chemicals which are non toxic, do not leave any residues and provide optimal antiseptic conditions) for minimizing cross contamination and eliminating the pathogens from hands [11]. Acquiring of correct hygiene habits for children are very important as well as the usage of correct chemicals for handwashing procecures [12]. Microorganisms in the hands due to their duration period are seperated into 2 groups as permanent and temporary microorganisms [13]. Soil and water originated permanent microorganisms as *S. aureus. B. cereus* and coliforms can induce food intoxications and the infections can be fatal [14]. Permanent pathogens generally use cross and animal/ soil - food - human digestive system contamination ways and grow in the fat tissue of the hands and the agents are continiously spread by the hosts unless an effective and continious handwashing processes are performed [13]. For these reasons, especially hand washing habits in children are very important for both food and public health.

According to our results, ages and genders of the students were not effective individual parameters on microbiological pollution of hands. These findings cause to think us habits acquired in the childhood period do not change. Gaining hand and general hygiene habits for persons, especially for children is very important for permanent and continious hygiene implementations.

Habits gained in early childhood period generally continue lifetime. In this aspect, in the case of gaining inadequate hyigiene habits of people in early period of childhood period could hardly be changed and this situation can be a serious risk factor. Another point that must not be forgotten is; a preschool student with inadequate hygiene habits can be a serious risk factor if he/she works at any food related position when he/she is adult for the total plant in spite of the mentioned risk factors have not been evaluated in our study. It can be very difficult changing the habits acquired, no matter how the food hygiene training enterprises/implementations are effective applied by the plants and/or institutions [1,15]. Because of the reasons explained above, it is so important to gain children very first general and hand hygiene habits for public health and food security chains.

Diaper usage variable was effective on total coliform bacteria parameter due to the results we got. Leakage of feces and urine through cloth nappies may lead to contamination of hands (both caretakers and children) and also the inanimate objects in the surroundings [16]. Because hand - to mouth behavior of the children, total coliform bactreria can be a risk factor for the kindergarten students who use diapers. Besides, food and/or surface contamination from hands may be used as a contamination way by total coliform group bacteria for contaminating the schools and the other students and this situation is evaluated as a serious risk factor for the schools, so for the other students. Mentioned possible transmission route of the agents may be a common cause for acqusition of diarrhoeal ilnesses in kindergatens. It has been documented that transmission of diarrhoea asscociated with total coliform group bacteria, *E. coli, Shigella* spp. and rotavirus in kindergartens and day care centers due to poor hygiene facilities [17]. In spite of it has not been investigated in our study, fecal contamination can also be responsible for transmission of hepatitis [18]. According to our results, the number of the coliform bacteria contaminated objects such as toys, activity tables and surfaces was significantly low in not - diaper using classes than the diaper using classes (data not shown). Altough modern age diapers are designed for maximal leakage prevention, hands of the caretakers and students can be a very important contamination source for coliform group bacteria if the adquate hygiene applications are not implemented.

Handwashing frequency variable was effective on all the microbiological parameters. In spite of hand washing frequency variable was first degree decisive for the

microbiological hand loads of the students, it must not forgotten that the mentioned variable's effect may vary according to the some secondary related variables as the time of handwashing process, usage of effective chemicals, hygiene conditions of common areas. Japanese Ministry of Health declared that 37% of total food intoxication cases in Japan were based on inadequate handwashing processes in the yaer 1998 [19]. Scott [20], indicates that this rate increase up to 80's % in Europe. Unfortunately there is not an exact rate decleration in our country but we assume that inadequate hand washing processes is a very important factor for food intoxication cases. Handwashing implementations are indicated as a very important preventive process for eliminating the pathogens which use faeces - hand - mouth/food contamination pathway by the medical literatures [21-24]. Correct handwashing implementations is very important to decrease the incidence of cross contaminations and help to protect total hygiene of the plants.

In our study, it was also examined school group differences (according to the economic income levels) with the idea of the possible effects of the variables to the schools. For this purpose each school was evaluated seperately and the group differences among the schools were exposed. Our study continued one school term (April - June). When the effects of seasonal factors to the hand microbioogical loads of students were investigated, it was determined that there were significant differences among all the parameters except *E. coli* (P<0.005). The significant differences were in favor of April which is a relatively cooler month than May and June due to the results we got. *E. coli* can easily grow in low temperatures (2 - 12°C) [25] and that is why we think that seasonal factors did not effect our *E. coli* parameter results significantly. Load of *E. coli* in the students' hands was not affected by the seasonal reasons and it is thought that this situation may pose a risk to students all the months of the year. Besides, growing ability of *E. coli* in low temperatures can be seious risk factor to the foods that are stored in refrigerators for both schools and houses. According to the results we got, the students of the schools which were located in the regions that had higher economic income level relatively were "cleaner" than the schools which were located in the regions that had lower economic income level relatively. All the chosen microbiological parameters showed a significant differences (P<0.05) due to the economic income variable. According to Dharod et al.[26] in United States America, the hand mincrobiological loads of the individuals who earn less than 1.000 USD/month were significantly higher than the individuals who earn more than 1.000 USD/month. Our results, too, were parallel to Dharod et al. [26] and we observed that there was an inverse rate between hand microbiological pollution of the students and economic status of the regions that the schools were located in. Medical literature indicates that hand washing frequencies of the families that have very nominal economic income

levels (300 USD and lower) are lower than it should be [26,27]. According to the results we got, it was determined that the handwashing frequencies of the students in the schools in low income districts were lower. Moreover, we observed that there were not soaps in the toilets of the schools included lower economic income group. We also observed that all the schools included to the high income level had hand - washing instructions in their toilets while one school included to the low economic income group had the mentioned instruction. It is thought that usage of ineffective disinfectants and/or absence of disinfectants can seriously increase the existance of pathogens in the hands so the risk factors for the schools.

Schools included to both low economic and high economic income levels were also compared among themselves seperately and the significant differences was only determined for the *S. aureus* parameter at the students of high economic income level schools. There were no significant differences for the other microbiological parameters analyzed for the shools included to both low and high economic income levels when the two school groups were compared seperately. This situation cause us to guess that *S. aureus* is an indicator microorganism for the students of the high economic income level schools. *S. aureus* is the only permanent foodborne pathogen of the hand microflora [28]. The agent can penetrate to the adipose tissue of the hands and can be eliminated only by using effective disinfectants with high frequencies in long times [29]. *S. aureus* can easily grow in almost all kinds of media however, generally, it can be found especially in the flora of nose, mouth and hands, and this situation facilitates of the cross contamination of the agent. According to the results we got, last hand loads of all investigated microbiological parameters except *S. aureus* were parallel for the students included into the similar economic income level group schools, however, the beginning flora of the hands of the students of different school groups were different. As a result, we can say that economical status is a very important and effective variable for the microbiological hand loads and cross contaminations of the foodborne pathogens.

Foodborne pathogens are widely seen all over the world and threat public health seriously. Altough the production of the foods under adequate hygienic conditions by the implementations of latest food security sytems, secondary contamination sources may be very important risk factors for even safely produced foods. Individual variables of people, especially children were determined as very decisive factors on the contamination of foods and schools in spite of the mentioned factors seem to be unrelated on foodborne pathogens contamination according to our results. Social and individual variables may be very effective hidden risk factors for the total contamination pathways of foodborne pathogens. It must not be forgotten that providing to gain the children

correct hygiene habits may decrease the incidence of foodborne pathogens and unpredictible/unmeasurable risks significantly.

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