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A Bacteriological Study of Mid day Meals Served and Hygiene Practices Maintained in Various Crèches of Chandigarh

KEYWORDS: Crèches, Microbial load, E.coli, Antibiotic sensitivity test.

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ABSTRACT *The present study was undertaken to assess the microbial load of mid day meals served at various 40 crèches of Chandigarh and nearby places. Food and swab samples were tested and E.coli was isolated from them. In various Crèches, total bacterial count of various food samples ranged between 9.3 × 103 CFU/ gm to 2.6 ×106 CFU/ ml, where as gram negative counts ranged from 1.1 × 102 CFU/ml to 7.9 × 106 CFU/ml. The counts in all types of crèches were within permissible limits. The results show highest values of mean for samples of water from various crèches. E.coli isolation was higher in swab samples (71.1%) as compared to food samples (46.6%). A total of 164 E.coli strains were isolated from various food and swab samples collected from different crèches, out of which 100 samples were tested for their virulence properties. E.coli isolates were subjected to Salt Aggregation Test (SAT) and Antibiotic sensitivity test. Chi square test was used and showed a significant relation between multi drug resistance and cell surface hydrophobicity. Hygiene practices followed by personnel in various crèches of Chandigarh and nearby places were surveyed. Knowledge of hygiene and sanitation was imparted through distribution of handouts and demonstration of effective hand washing steps to the workers in all the crèches*

INTRODUCTION

Children are the future of the nation and they have to be nurtured in a healthy atmosphere with proper food and education. Child care can also provide support services for working parents. Child care can enhance children's social and emotional development and also a provision of nurturing relationships, a stimulating environment, and basic health and safety[1]. Child care centres are operated by non-profit organizations or parent co-operatives, private companies or individuals. Mid-day meals are also provided regularly to these children as part of the nutritional support programme of Indian Council of Child Welfare Crèches[2]. Every day, more than 69 lakh children take food and supplementary nutrition in the Noon Meal and Child Centres[3].

Infection in children in child day-care centres has been a concern since the 1980s. In a study, it was put forth that the children in residential child care centres were at a higher risk of infectious diseases with respiratory tract infection being the most common infectious disease in these centres[4]. Infections can spread easily because germs can be present in faeces, saliva or nasal secretions, on skin, or sometimes in the eyes of an ill person. Young children tend not to wash their hands without supervision and they have poor personal hygiene habits[5]. Another study showed that the incidence rates for diarrhoea and colds were highest in children from child day care centers[6].

E.coli are the commonest type of organisms which are reported to be growing in different types of food and their presence as an enteropathogenic strain has been implicated in almost all cases of infantile diarrhoea from different parts of India[7]. The infection with E.coli O157:H7 follows ingestion of contaminated food or water, or oral contact with contaminated surfaces[8]. Sanitation is particularly important in day care settings. The quality of food which is served in day care centres, hygiene where it is prepared and effectiveness of method of keeping it for a long time can be examined by microbiological examination. Therefore the present study was undertaken to highlight the major routes and extent of contamination in day care centres and attitude and behaviour of child care providers towards hygiene and sanitation.

MATERIALS AND METHODS

The study was conducted to determine the various sources of contamination and the microbial load of food samples served as Mid Day Meal in various Day Care Centres of Chandigarh.

Selection of Samples

Forty different day care centres from different sectors of Chandigarh and nearby places were visited. These crèches visited were being run by the Indian Council of Child Welfare (ICCW), Chandigarh Social Welfare Board (CSWB) and privately owned crèches. The various food and water samples from the mid day meals were collected. The swabs from the hands of children, hands of food handlers /workers and work surfaces were also obtained. A questionnaire on hygiene and cleanliness practices followed at the day care centres was made and filled in by interview method. The caretakers/teachers were interviewed regarding the hygiene practices followed by them. They were given handouts on hygiene and sanitation to impart knowledge about- Steps of hand washing, Advantages of boiling water, Methods of rodent control, Hazards of the use of plastic and steel in microwave oven and cleanliness and sanitation techniques to be used in crèches

Determination of Microbial Load of Food Samples

Standard plate count technique was used to determine the total and gram negative counts of the samples. The various media used were- Nutrient agar for the total bacterial count and Mac Conkey's agar for the gram negative count.

Isolation of E. coli

The swabs, food samples and water samples were streaked on Mac Conkey's agar and the non-mucoid lactose fermenting colonies were picked up and cultures purified by the plate streak method on EMB agar.

Identification of Isolates

The purified cultures were identified on the basis of colony

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morphology, gram staining and specific biochemical tests. Classification of bacteria was done based on Bergey's Manual of Determinative Bacteriology, 8th edition[9].

Bacterial Cell Surface Hydrophobicity[10]
This test was conducted to determine the hydrophobicity of E.coli isolated from food samples and swab samples from possible sources of contamination.

Antibiotic Sensitivity Test[11]
E.coli isolates were tested to determine its susceptibility against different antibiotics.

Data Analysis
In order to determine the correlation between multi drug resistance and cell surface hydrophobicity the chi-square test was applied.

RESULTS AND DISCUSSIONS
A total of 260 Samples of various foods served and swab samples of working area, hands of the worker and children were procured from these forty day care centres. Amongst these 40 day care centres, 20 were being run by Indian Council of Child Welfare (ICCW) and providing one mid day meal to children. Ten privately owned crèches did not provide any meal therefore two tiffin food samples were procured in the place of mid day meal from such crèche. Ten crèches being run by Chandigarh Social Welfare Board (CSWB) provided one mid day meal.

Determination of microbial load of food and water samples was undertaken to assess the number of microorganisms present in the food and water being fed to the children. The total count was taken for a variety of foods procured during the study. Table 1 shows the range of total bacterial count and gram negative counts taken from various food samples collected from various crèches of Chandigarh and nearby places. However, the gram negative count in case of private crèches was lesser as compared to other crèches.

Table 1: Total bacterial and gram negative bacterial load range in samples collected from various crèches.

Crèche	Samples	Total bacterial count (CFU/gm/ml)	Gram negative count (CFU/gm/ml)
ICCW CRECHES	FOOD AND WATER	2.6×10^4 – 3.62×10^5	1.0×10^3 – 1.11×10^2
PRIVATE CRECHES	FOOD AND WATER	4.8×10^5 -- 4.22×10^4	2.4×10^2 --- 3.86×10^2
CSWB CRECHES	FOOD AND WATER	2.4×10^5 -- 4.12×10^4	1.1×10^2 --- 1.64×10^3

Isolation of E.coli from various food samples taken from various crèches is shown in Table 2. A total of 90 food samples and 170 swab samples were collected from these crèches out of which 42 food samples and 121 swab samples showed the isolation of E. coli. Minimum isolation of E.coli from food samples was seen in ICCW crèches. Highest E.coli isolation (84%) from swab samples was noticed in CSWB crèches. These crèches had very unhygienic and poor sanitary environment for the children.

Table 2: Number and percentage of E.coli isolated from various samples collected from crèches in Chandigarh.

Type of crèche	No of crèches	Total number of samples		Number of E.coli isolated		Percent isolation of food samples	Percent isolation of swab samples
		Food	Swab	Food	Swab		
ICCW	20	40	80	13	53	32.5 %	66.2%
Private	10	30	40	15	26	53.3%	65%
Social Welfare Board	10	20	50	13	42	65%	84%
Total	40	90	170	42	121		

Antibiotics are chemical agents that interfere with the growth and activity of microorganisms and are used to prevent transmission of various infections. But nowadays, Antibiotic resistance reaching the population is a serious problem to human health. Thirty E.coli strains from food samples and 70 E coli strains from swab samples, that is, a total of 100 strains were subjected to the Antibiotic sensitivity test. Table 3 shows that the maximum sensitivity of E.coil strains from food and swab samples were shown towards Chloramphenicol (87%) followed by Erythromycin (82%), Gentamycin (68%) and Nalidixic acid (50%) whereas minimum sensitivity has been noticed towards Ampicillin (27%).

Possession of R-plasmid as a wide spread phenomenon in the most of the Enterobacteriaceae is reported to be the major cause of multi drug resistance.[12] In this study, multi drug resistance (resistance to one or more antibiotics) was observed in 54% of E.coli isolates gathered from food and swab samples collected from various crèches. Table 3 shows the distribution of multi drug resistance strains isolated from various mid day meal, tiffin foods, and water and swab samples. Out of100 strains are tested, 20 were not resistant to any antibiotics, 25 were resistant to one antibiotics, 17 were resistant to two antibiotics, 20 were resistant to three antibiotics, 16 were resistant to four whereas only one were resistant to five antibiotics.

Hydrophobicity is the chief component to determine an organism's pathogenicity. A total of 100 E.coli strains were tested for determining the incidence and degree of cell surface hydrophobicity and 93% strains gave a positive SAT test. The 48% bacterial cells showed aggregation even at minimum concentration of 0.02 M Ammonium sulphate.

Table 3: Antibiotic Sensitivity of E.coli isolates from various sources of contamination and food samples and Multidrug Resistance of 100 E.coli isolates

ANTIBIOTICS	PERCENT SENSTIVITY OF E.coli (%)	Resistant against no. of Antibiotics	Percentage of E.coli exhibiting resistance (%)
Ampicillin 35 µg	27	None	20
Gentamycin 30 µg	68	One	25
Erythromycin 30 µg	82	Two	17

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Chloramphenicol 30 µg	87	Three	20
Nalidixic acid 30 µg	50	Four	16
		Five	1

A total of 100 strains of E.coli were subjected to the salt aggregation test (SAT) for determining the incidence and degree of cell surface hydrophobicity. Out of 100 strains 48 were highly hydrophobic, 14 optimally hydrophobic, 18 moderately hydrophobic, 13 had minimum hydrophobicity while 7 strains were not at all hydrophobic. All strains of E.coli tested indicated high degree of hydrophobicity. Hence this property shows a better opportunity to pathogens for attachment[13]. Table 4 represents the percent distribution of E.coli from samples according to their cell surface hydrophobicity, taken at varying concentrations (0.02M, 0.2M, 1.8M, 3.2M) of ammonium sulphate. With the increase in concentration of ammonium sulphate, the rates of hydrophobicity also increased.

Table 4: Surface Hydrophobicity of E.coli Isolates from Food and Swab Samples of Various Crèches at Varying Concentration of (NH4)2SO4

Samples	No. of E.coli Isolates	No. of E.coli isolates showing aggregation at different concentrations of $(NH_4)_2SO_4$			
Total Samples	100	0.02 M 48	0.2 M 62	1.8 M 80	3.2 M 93

Table 5 showed a significant correlation between multi drug resistance and cell surface hydrophobic organisms at 39.045 chi square value. This implies that those two characters may be located on the same plasmid as all these properties are plasmid mediated.

Table 5: Correlation of multidrug resistance with cell surface hydrophobicity

Total no of strains= 100	Yes	No	Chi square value	Df	p Value
No. of Multi Drug Resistant Strains	54	46	39.045	1	.0001
Cell Surface Hydrophobicity	93	07			

p value ‹ .001 = * i.e. Significant**

A questionnaire on hygiene practices maintained in day care centres was filled in by the interview method. In this study, we found minimum trained personnel (in food hygiene) in CSWB crèches.

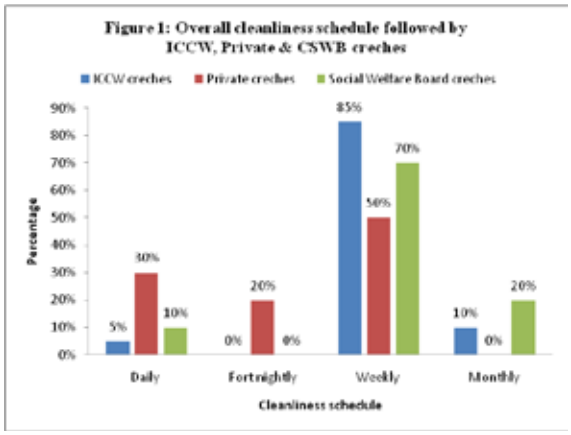

Figure 1 shows the comparison between ICCW, Private and Chandigarh Social Welfare Board crèches on the basis of their overall cleanliness schedule. Maximum crèches were following weekly overall cleaning schedule. Therefore, Private Crèches were the best crèches on cleanliness basis as compared to those run by ICCW and CSWB. They maintained better cleanliness, hygienic conditions. The poorest hygiene and sanitation conditions were noticed in CSWB crèches as the personnel were not educated and trained. The crèches being run by ICCW had better sanitary conditions and environment than the CSWB crèches.

SUMMARY AND CONCLUSION

Thus, in our study, a significant difference was observed in the bacterial counts of food which the children brought from their home and the foods which were prepared in the ICCW crèches. This implies that the cooks at the ICCW crèches were equally aware of the right methods of cooking and took proper care in cooking foods. The results show that the total bacterial and the gram negative count in case of private crèches was more as compared to the other crèches due to improper storage conditions. The ICCW and CSWB crèches served freshly cooked food, hence, the high counts in them could be attributed lack of hygienic methods used for cooking the food, or improper washing of hands of handlers. Therefore, during our data collection, we imparted knowledge of hygiene and sanitation through distribution of handouts and demonstration of effective hand washing steps. At the end we conclude that, there is an urgent need to recognise good, healthy, hygienic environment to reduce illness and enhance care and health of children at day care centres of different organisations by implementing best hygiene practices.

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