

Prevalence of *B. cereus* in
“Chinese Style’ fried rice available
in hotels, restaurants and
take away outlets in Colombo City

By

MANOSHA LAKMALI PERERA

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ABSTRACT

B. cereus is an environmentally ubiquitous, Gram positive, facultative anaerobic, motile, spore forming bacillus responsible for two distinct types of food poisoning syndromes as well as other manifestations of pathogenicity.

The objectives of the present study were to find out the prevalence of *B. cereus* in “Chinese style” fried rice available in hotels, restaurants & takeaway outlets in Colombo City, to evaluate the association of selected risk factors and the contamination of fried rice with high *B. cereus* count ($> 10^6$ cfu/g), to identify the potential serotypes involved which can cause food poisoning in Colombo city, to study the antibiotic sensitivity patterns of the food isolates $> 10^6$ cfu/g of *B. cereus*, to assess hygienic quality of Chinese style fried rice available in hotels, restaurants and take away outlets in Colombo city, in terms of Aerobic Plate count, Coliform count, Faecal coliform count & E. coli count.

A total of 200 samples of “Chinese style” fried rice were analysed over a period of one year for enumeration of *B. cereus*. Using systematic random and cluster sampling technique, 25 eating places were selected in the Colombo city limits, in which “Chinese style” fried rice was prepared and available for sale. Structured questionnaires were used to collect data from these 25 eating places on their various practices on preparation and storage of “Chinese style” fried rice. These data were

collected by a trained interviewer. Data processing and analysis was done using SPSS 10.01 statistical software programme.

The total of 200 samples analysed in the present study consisted of 35% of vegetable variety, 29.5% samples of chicken variety, 18% samples of sea food variety, 9% samples of egg variety, 5% samples of beef variety, and 3.5% of mixed variety respectively.

Enumeration of *B. cereus* of fried rice was carried out using 0.1% peptone water as the diluent and polymyxin B. – egg yolk – mannitol bromothymol blue agar (PEMBA) / Bacillus cereus selective agar base (Oxoid code : CM 617) with *B. cereus* selective supplement (oxoid cod: SR 99), according to Oxoid Manual, 1990.

Confirmation & differentiation of *B. cereus* from the rest of the Bacillus species of Group I was carried out using biochemical tests and rapid confirmatory staining procedure and results were compared with reference strain of *B. cereus* (NCTC 008035), according to Oxoid Manual, 1990, SLS 516: part 8: 1983 standard & USFDA Bacteriological Analytical Manual online, 2001.

Serotyping of *B. cereus* isolates, obtained from fried rice having counts $> 10^6$ cfu/g were carried out at food safety Microbiology Laboratory, PHLS Central Public Health laboratory, Collindale NW, U.K. due to unavailability of antisera commercially.

The antibiotic susceptibility of *B. cereus* isolates obtained from fried rice having counts $> 10^6$ cfu/g using antibiotics (Viz, ciprofloxacin, erythromycin, gentamicin, penicillin, chloramphenicol and amikacin) was carried out by Joan Stoke method.

Aerobic plate count was carried out using USFDA Manual of Food Quality Control, 1992. Detection and enumeration of coliforms, faecal coliforms and *E. coli* were carried out using SLS 516: part 3: 1982 standard.

Out of 200 "Chinese style" fried rice samples analysed *B. cereus* was detected in 112 (56.00%) of samples. Twenty eight (14.00%) of the total number of samples (200) contained more than 10^6 cfu/g which is generally considered as the infectious dose. Moreover highest prevalence of significant colony count ($> 10^6$ cfu/g) was seen in Chicken variety 14 (23.73%). Five (35.71%) of chicken variety samples were contaminated with *B. cereus* colony count of $> 10^6$ cfu/g were in the range of $10^9 - 9.9 \times 10^9$ cfu/g. None of beef variety fried rice contained *B. cereus* $> 10^6$ cfu/g. Of the total No: of samples (200) analysed 42.00% samples contained $\leq 10^6$ cfu/g. Highest prevalence in this category were seen in sea food & egg varieties both having (44.44%). Beef variety had lowest prevalence in this category that is (20%). According to the results almost half 40, (47.62%) in all varieties except beef, *B. cereus* colony count was in the range of $10^5 - 10^6$ cfu/g.

Highest percentage (80%) of samples in which *B. cereus* was not detected belonged to the beef fried rice variety and 48.57% of vegetable variety too did not contain *B.*

cereus. There were statistically significant associations between selected risk factors namely **Temperature (room temperature) of storage of boiled rice, Time (> 4hrs) of storage of boiled rice at room temperature, before frying and frequency of cooking (more than once) of rice for lunch** with high *B. cereus* count $>10^6$ cfu/g in fried rice after controlling for confounding factors. Type of rice and amount of rice boiled per day were not significantly associated with high *B. cereus* count $>10^6$ cfu/g. Moreover time of storage more than 4hrs at room temperature after frying was not independently associated with high *B. cereus* count ($>10^6$ cfu/g) of fried rice.

Serotypes of *B. cereus* isolates obtained from fried rice samples $>10^6$ cfu/g as follows: 53.57% were not typable. Most predominant serotypes were H. 15 & H. 19 both had similar prevalence of 14.29%. Serotypes H. 20, had prevalence of 10.71%.

Antibiotic Sensitivity pattern of *B. cereus* isolates obtained from fried rice having counts $> 10^6$ cfu/g, showed the same sensitivity pattern 100% sensitivities were obtained to antibiotics such as ciprofloxacin, erythromycin, gentamicin, chloramphenicol and amikacin. 100% resistance was seen to penicillin.

Moreover 39.50% of the total fried rice samples (200) tested contained and aerobic plate count $>10^6$ cfu/g, 24% of samples contained $\geq 1,100$ Coliforms /g of rice. 16% of the total fried rice samples (200) tested contained $\geq 1,100$ faecal Coliforms /g. *E. coli* was detected in 39% of the fried rice samples tested and the highest percentages of positive samples were found in egg, vegetable and mixed varieties.