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Summary

Spices, that are capable of transforming an ordinary meal into an adventure, are important vehicles for carrying various micro-organisms implicating possible health problems for consumers and shelf-life problems for foods. This project was undertaken with the objective of evaluating microbiological quality of spices retailed in Indian markets and understanding some behaviour pattern of the pathogenic bacterial isolates from spices. A total of 154 samples of 27 kinds of spices collected from retail shops of 20 States of India was investigated to determine their microbial status. Following the ICMSF (International Commission on Microbiological Specifications for Foods) guideline, the total aerobic mesophilic bacteria (TAMB) count showed that 51% of the samples were in the unacceptable level (>log 6 cfu g^{-1}).

Bacillus cereus, Clostridium perfringens, Staphylococcus aureus and members of Enterobacteriaceae occurred in 85, 59, 11 and 85%, respectively of the kinds. Black pepper powder, caraway, garlic and red chilli did not contain *B. cereus*. On the other hand, this aerobic sporeformer was found in all the samples of ajmud, small cardamom and cumin powder. Coliforms and faecal coliforms were found in 33 and 15%, respectively of the kinds. *Escherichia coli* was detected in only one sample, of garlic. *Salmonella* and *Shigella* were found only in 2.6% of the samples. Although the non-packaged spices contained less count of TAMB, those had a higher load of *B. cereus* and Enterobacteriaceae compared to polyethylene-packaged ones.

Strains of foodborne bacterial pathogens that are resistant to a variety of antimicrobial agents have become a major health concern. The extent of prevalence of antimicrobial resistance among the spice isolates was determined. The tested strains of *B. cereus, Cl. perfringens, Staph. aureus, E. coli, Salmonella* and *Shigella* were found resistant to at least three antimicrobials.

The D-values and z-values for spores help to understand the hazardous potential of these organisms which can survive the cooking processes. In brain heart infusion broth supplemented with glucose(BHIG), the $D_{100°C}$-values for *B. cereus* were 3.5-5.9 min, and the z-values were 17-18°C. The $D_{100°C}$-values for *Cl. perfringens* in fluid thioglycolate medium ranged from 10.5 to 18.5 min.

Bacillus cereus enterotoxin (BCET) and *Cl. perfringens* enterotoxin (PET) were estimated using reversed passive latex agglutination method. Seventy-four percent of the tested 23 strains of *B. cereus* were able to produce 8 to >256 ng BCET ml^{-1} BHIG; 60% of the strains produced 32 ng BCET ml^{-1}, two strains produced 128 ng BCET ml^{-1}, and only one strain at 256 ng BCET ml^{-1} level. Of the selected 16 strains, 19% produced 2-32 ng PET ml^{-1} modified Duncan Strong medium. Some market samples too, like cumin powder contained a high BCET titre (64 ng g^{-1}).

After intentional inoculation of black pepper powder (where none of the five samples contained *B. cereus*) with an enterotoxigenic strain of *B. cereus* and 14 d-incubation at room temperature, there was no significant ($P<0.05$) change in the cell count and BCET production. This indicates that a spice can support the survival of pathogenic organism and act as a vehicle for contaminating foods.

To assess safety of spicy foods, *aloo dam* (a potato-based popular food) and goat meat gravy were taken as experimental subjects for the respective growths of *B. cereus* and *Cl. perfringens*. Freshly prepared *aloo dam* did not contain *B. cereus*, however small cardamom acted as a carrier when the food was seasoned with it. Immediately after seasoning, the initial count of *B. cereus* cells was log 2.7 g^{-1} and the BCET content was 8 ng g^{-1}. After keeping the food at room temperature (27-30°C) for 21 h, the cell count increased by four log cycles and the BCET content raised to 128 ng g^{-1}. After intentional inoculation with enterotoxigenic *B.*

cereus 120-B1 (initial count, log 3.5 cfu g^{-1}; BCET, 16 ng g^{-1}) and incubation at room temperature for 21 h, the cell count and BCET content increased by 4 log cycles and 16 times, respectively. An enterotoxigenic strain of *Cl. perfringens* multiplied rapidly in the gravy; after 19 h the cell count increased by more than 4 log cycles however the PET content (2 ng g^{-1}) remaining unchanged. After boiling the 19 h-long incubated gravy for 15 min on a water bath, the cell count fell down to log 3.1 cfu g^{-1} and the PET content went below the limit of detection. It was confirmed that spices, when added to foods, are able to support outgrowth of bacterial pathogens and production of enterotoxins.

A novel convenient technique for primary screening of bacterial susceptibility to garlic, using a slice from its clove, showed a high sensitivity (71%) towards *B. cereus*. Aqueous extracts of garlic were found to possess a potent bacteriostatic principle active against both Gram-positive and Gram-negative bacterial pathogens *in vitro*. Minimum inhibitory concentrations (MICs) of garlic in extract were 6-10 mg g^{-1} for *B. cereus*, 20 mg g^{-1} for *Cl. perfringens*, 30-40 mg g^{-1} for *Staph. aureus* (excepting the isolate from garlic, where the MIC was 100 mg g^{-1}), 10 mg g^{-1} for *E. coli* (30 mg g^{-1} for the garlic isolate), 40-100 mg g^{-1} for *Salmonella*, and 10-40 mg g^{-1} for *Shigella*. It inhibited the growth of all these strains which were resistant to a number of commonly used antibiotics. The production of enterotoxin (diarrhoeal type) by *B. cereus* was 98% inhibited at 9 mg garlic g^{-1} extract (sub-inhibitory concentration).

All the representative isolates from spices grew optimally at near-neutral pH (6.61-7.51). The MICs of sodium chloride were 45-80 mg ml^{-1}. The MICs of benzoic acid for *B. cereus*, *Staph. aureus*, *E. coli*, *Salmonella* and *Shigella* were 250-800 μg ml^{-1}, whereas for *Cl. perfringens* it was 1900-2200 μg ml^{-1}. Similarly, the MICs of sorbic acid for all the tested isolates, excepting *Cl. perfringens*, were 600-1100 μg ml^{-1}. Of the eight isolates of *Cl. perfringens*, only three were inhibited at 2000 μg ml^{-1}, while others were resistant. Sixty percent and 75% of the respective strains of *B. cereus* and *Cl. perfringens* were resistant to 5000 IU Nisaplin ml^{-1}, whereas the MIC values of *Staph. aureus* were between 3000 and 5000 IU ml^{-1}. The effect of combination of pH, sodium chloride, benzoic acid and nisin on the growth of 120-B1 was investigated. The best combination found for the cessation of growth was pH 6.2, 25 mg sodium chloride ml^{-1}, and 2000 IU Nisaplin ml^{-1}.

The present study demands spice cleanliness, commercial sterilization, proper storage and marketing to provide consumers a safe and quality produce.