

ISOLATION AND IDENTIFICATION OF SOME PATHOGENIC MICROBES FROM SUPERMARKET CARTS AND BASKETS IN RIYADH, SAUDI ARABIA

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ABSTRACT

Shopping carts and grocery baskets from 15 different supermarkets and grocery stores around Riyadh City, Saudi Arabia were randomly swabbed from samples for potentially harmful pathogens. Samples were inoculated into appropriate culture media; the single colonies of the isolates were identified by microscopic morphology, biochemical and physiological tests. The API 20E system was used in the further identification of the isolated micro-organisms grown on BAP. The bacterial isolates were *Bacillus* spp., *Corynebacterium* spp., *Klebsiella ozaenae*, *Serratia marcescens* as well as *Aspergillus* spp. as a fungal isolates. All these micro organisms are potential pathogens lurking in the otherwise "clean" shopping equipments. Further attention has to be done by appropriate authorities aside from the hygienic and precautionary measures by the shoppers themselves through efficient handwashing especially after shopping and handling shopping equipments.

INTRODUCTION

People usually often visit the supermarkets and grocery stores to purchase items essential to daily life. However, we are not mindful enough to know the presence of harmful bacteria living on surfaces in these public places. There is little scientific evidence to support the claim the presence of these microbes which can be harmful to us.

According to a study done by the Korean Consumer Protection Board (KCPB), shopping cart handles were found to have the largest colonies of bacteria with 1,100 colony-forming units (CFU) of bacteria per 10 cm². This is even filthier than a computer mouse, hand straps of buses, bathroom door handles and even toilet seats (Rahn,2006).

Pathogens have been known to be present in products found in the supermarkets. Literatures showed the presence of fecal coliforms in vegetables (Amoah, *et al.* 2006; Park and Sanders,1992), *Escherichia coli* in retail foods (Johnson *et al.* 2005; Estrada-Garcia, 2004), *Vibrio* spp. in seafoods (Elhadi *et al.* ,2004), *Listeria* spp. in raw poultry and fish products (Vitas and Garcia-Jalon,2004; Miettinen *et al.* ,2003), *Salmonella* in dairy and fresh meat products (Nassib *et al.*, 2003; Berglezova *et al.*, 1999; Rasul *et al.*,1996; Wray *et al.*, 1991) and *Clostridium botulinum* from fishes (Haq and Sakaguchi, 1980).

The incidence of human infection by these harmful pathogens from wet and dry produce from the supermarkets may be addressed by good quality control and supervision by the store administrators and health sanitary inspectors. Each locality has its own preventive guidelines implemented to ensure public safety in the purchase and consumption of these products

brought from the supermarket. But what is troublesome is when these harmful pathogens are present in surfaces such as supermarket shopping cart handles and baskets which are not frequently monitored and sanitized by authorities. These would expose innocent people from harboring these harmful pathogens. Therefore the aim of this investigation is to isolate and identify the pathogenic microbe/s present in these surfaces from different supermarkets in Riyadh City, Saudi Arabia.

MATERIALS AND METHODS

Setting: Fifteen different supermarkets and grocery stores in Riyadh City, Saudi Arabia.

Collection of samples: From each supermarket or grocery store, at least 3 samples were taken from shopping cart handles using a sterile cotton swab. For bacterial isolation each swab was placed in Nutrient liquid media which supported the growth of microbes and were incubated at 37°C for 24 to 48 hours and Sabouraud's agar media was used for the isolation of fungi.

Isolation and Identification of microbes:

After 24-48 hours of incubation, the colonies that appeared visually dissimilar were chosen, counted, transferred to fresh Blood Agar Plates (BAP) and incubated at 37°C for 24 hours. Identification of the microbial isolates did not commence until it was evident that a pure culture had been obtained. After 24 hours, colonies were observed for size, texture, color and hemolysis reactions. Colonies are Gram stained and individual bacterial cells were observed under the microscope.

Further needed analysis were done by 1-2 colonies from these plates and a standard isolate was made which were done using 0.5 ml McFarland barium sulfate No. 3 standard to quantitate the solution.

The major identification tool used for bacterial isolates was the API 20E system (Analytical Profile Index, BioMerieux, Durham, NC, USA). The procedure was carried out by using a sterile pipette, the bacterial suspension was inoculated to rehydrate each of the wells making sure that the end of the pipette touched the end of the cupule, allowing capillary action to draw the fluid into the well as bulb was slowly squeezed. This eliminated the possibility of bubble formation in the wells. Inoculation of specific test wells was done according to the manufacturer's instructions. The strips were incubated for 18 to 24 hours at 37°C. Results were read against the API 20E chart to identify the microorganism. Growths on Sabouraud's agar were identified microscopically.

Many other additional tests were done for further identification of the isolated microorganism. Through the additional use of Bergey's Manual (John,1994), Starr and Stolp (1981) , Leifson (1960), Lighthart and Loew (1972), Collins and Lyne (1976), Ward *et al.*,(1986), Atlas *et al.*,(1988) and Labeda (1990).

RESULTS AND DISCUSSION

Growth was observed after 24 hours incubation on BAP which revealed both Gram positive and Gram negative rods by Gram staining. In the identification of Gram positive rods, they were predominantly found to be aerobic, catalase positive *Bacillus spp.* and *Corynebacterium spp.* Identification of the Gram negative rods present by the API 20E system revealed *Corynebacterium spp.*, *Klebsiella ozaenae* and *Serratia marcescens*. *Aspergillus* was isolated and identified microscopically through its distinct hyphae with frequent branching septae.

The results that our study revealed appear suitably worrying. In accordance with the findings of the Korean Consumer Protection Board, shopping cart handles contain invisible microbes that can do serious harm to the population.

Generally, *Bacillus* species are neither morphologically nor phylogenetically indistinguishable from each other. Though most of the members of this genera are considered contaminants, there are 2 members which are of significant medical importance, *B. anthracis* and *B. cereus*. *B. anthracis* causes anthrax and *B. cereus* causes food poisoning.

Corynebacterium infection usually causes toxic manifestations involving the heart, kidneys and nerves brought about by the toxins released by the bacteria to the bloodstream. Mortality rates are very high especially if it affects the kids and the elder people.

Klebsiella ozaenae causes ozena, associated with upper respiratory tract infection. Initially presenting with atrophic rhinitis, it can progress to a full-blown infection of the lungs. Lately, *K. ozaenae* has been implicated to cause urinary tract infection (UTI) soft tissue infection (Goldstein *et al.*, 1978), corneal abscess (Janda *et al.*, 1978), meningitis and septicemia (Lewis *et al.*, 1979), cystic fibrosis (McCarthy and Hubbard, 1984) and cerebral abscess.

Serratia marcescens, the only pathogenic specie of *Serratia* causes about 2% of nosocomial infection of the bloodstream, lower respiratory tract, urinary tract, surgical wounds and skin and soft tissue infections. Outbreaks can cause meningitis, endocarditis and osteomyelitis. Mortality is around 26% and is even higher when it presents with meningitis and endocarditis.

Aspergillus infection ranges from allergic bronchopulmonary aspergillosis to the more invasive infections of the lungs and the central nervous system (CNS). Mortality is high (50% to 100%) when it goes invasive causing endocarditis and renal abscess.

Our study showed that these potentially harmful pathogens are easily accessible to every individual through handling of contaminated sources such as shopping cart or a grocery basket. Most people are caught unaware that these pathogens are "lurking" invisibly in the otherwise "clean" appearing shopping cart handles. Still, the only sure way to annihilate these possibly harmful bacteria picked up at the supermarket is by washing with soap and water frequently after every shopping.

Conclusion Potential harmful pathogens are picked up at places and surfaces frequented by people such as a shopping cart handle or a grocery

basket. Gram positive rods such as *Bacillus* and *Corynebacteria* and the Gram negative *Klebsiella* and *Serratia* and the fungi *Aspergillus* can be transmitted from these places and cause infections in humans with moderate to high mortality rates.

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عزل وتعريف بعض الميكروبات المرضية الموجودة على عربات وسلات التسوق في مراكز التسوق بالرياض- السعودية
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تم جمع عينة عشوائية من مواضع مسك عربات التسوق من خمسة عشر بقالة وسوق مركزي في مدينة الرياض السعودية ، وقد نميت الميكروبات على بيئات مناسبة وعزلت في صورة نقية ، وقد تم تعريفها مبدئياً بالفحص المجهرى للشكل الظاهري ، والاختبارات البيوكيميائية . ثم استخدم نظام API 20E في ما بعد في التعرف على العزلات المختلفة. والأنواع البكتيرية التي تم عزلها وتعريفها هي *Bacillus* spp., *Corynebacterium* spp., *Klebsiella ozaenae*, *Serratia marcescens* و الفطر *Aspergillus* spp. . جميع هذه الميكروبات المسببة للأمراض متواجدة كامنة في عربات التسوق . لذلك لا بد من مزيد من الاهتمام من قبل المتسوقين وأخذ التدابير الاحترازية من خلال غسل الأيدي بعد التسوق وملامسة تلك العربات.

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