Enteric Bacterial Contamination in Ready to Eat Food Products and their Resistance to Commonly Used Antimicrobials

Sukhminderjit Kaur, Divya Walia

Department of Biotechnology,
Shaheed Udham Singh College of Research and Technology, Tangori, Mohali.

ABSTRACT

Background & Objectives: There is an increasing tendency among people for the consumption of ready to eat food, especially among urban people, which increases the risk of food-borne diseases. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades. The present study was conducted to isolate various enteric bacterial pathogens from ready to eat food samples sold in local market and to check their susceptibility to commonly used antimicrobials.

Material & Methods: For the isolation of enteropathogens, 50 different ready to eat foods were collected from retail market of Chandigarh, India and its periphery. The samples were inoculated on MacConkey agar after serial dilutions and isolates obtained were identified morphologically and biochemically using standard procedures. Their susceptibility to commonly used antimicrobials was checked.

Results: A total of 57 bacterial isolates were obtained which included *E.coli* (42%), *Klebsiella spp.* (25%), *Salmonella* spp. (9%), *Enterobacter spp.* (9%), *Pseudomonas spp.* (5%), *Shigella spp.* (4%) and *Proteus spp.* (4%). The isolates showed highest rate of resistance towards amoxicillin followed by norfloxacin and nalidixic acid.

Interpretation & Conclusion: All of the isolated bacteria showed resistance to two or more antibiotics studied. The prevalence of antibiotic resistance among food-borne pathogens is a major threat which may pose difficulty in further treatment.

Keywords: Antibiotic resistance, enteropathogens, food borne pathogens

INTRODUCTION

As a new trend of eating habit, the people increasingly prefer to take foods from outside in the form of ready to eat food. If food products are not handled carefully, then there are chances of various types of food borne illness. Food- borne illness associated with the microbial pathogens or other food contaminants is a serious health threat in developing countries. Food borne illnesses is caused by many harmful bacteria, viruses, parasites or chemical contaminants of the food, Bacterial agents remaining the leading cause of such diseases.[1] Raw foods including meat, poultry, fish & shellfish, eggs, unpasteurized milk and other dairy products can contain enteric bacteria due to sewage contamination. These bacteria can multiply exponentially at any time during processing, storage, and packing making food harmful to eat.

Diarrheal disease is one of the most important cause of morbidity and mortality among children in developing world.[2] The World Health Organization (WHO) has estimated that 70% of diarrheal episodes are caused by biologically contaminated food.[3] About 1.5 billion global episodes of diarrhea occur annually mainly in developing countries, resulting in 3 million deaths among children of less than 5 years of age. Globally mortality estimates from diarrhea and its complications range from 1.5 to 5.1 million deaths per year for children

Corresponding Author :
Dr. Sukhminderjit Kaur, Assistant Professor
Department of Biotechnology,
Shaheed Udham Singh College of Research and Tech.
Tangori, Mohali. Pin: 140 306
Phone: 01762 306152
Email: sukhminderphd_6@yahoo.co.in
Fax: 01762 306123

under the age of five.[4]

Antimicrobial resistance among food-borne pathogens has emerged as a serious problem during recent decades. Antibiotic resistance is recognized as a growing problem that poses a major threat to the continued effectiveness of antibiotics used to treat human illness. If antibiotic resistance confers resistance to antimicrobials used in the treatment of disease in man, the infection may become difficult to treat. Pharmaceutical companies are developing fewer new antibiotics to replace those that are no longer effective.[5] *Salmonella spp.* is the most common cause of antibiotic- resistant outbreaks identified in different studies.[6,7] This increase in antimicrobial resistance is attributed to the selection pressure created by using antimicrobials in food-producing animals, in addition to the unregulated use of antibiotics by humans in developing countries.[8]

In view of the above mentioned facts, the present study was undertaken to isolate various enteric bacteria from different ready to eat foods sold in local market and to check their susceptibility to commonly used antimicrobials.

MATERIALS AND METHODS

Different ready to eat food samples viz. burger, muffins, pudding, bread, fruit cake, hotdog, pastries, sandwich, cream roll etc were collected from retail market of Chandigarh, India and its periphery. After collecting the food item, it was immediately transferred to Microbiology Laboratory of Shaheed Udham Singh College of Research and Technology, Tangori, for further processing. For the isolation of enteropathogens, the food samples were inoculated on MacConkey agar (MA) (HiMedia, Mumbai). The plates were incubated at 37°C for 24 – 48 hrs. The plates were observed for lactose fermenting and non lactose fermenting colonies. The enteric isolates were identified further by various morphological and biochemical tests In order to determine the sensitivity or resistance of the enteric pathogens against various antibiotics, Kirby Bauer disc diffusion method was performed.[9] Freshly prepared Muller-Hinton agar (HiMedia, Mumbai) plates were seeded with the culture. Various antibiotic disks were dispensed on the cultured plate by gently pressing the disk with the sterile forceps. Plates were then incubated at 37℃ for 24 h. Antibiotics like norfloxacin, nalidixic acid, ciproflaxin, and amoxycillin were used. After incubation, the diameter of the zone of inhibition around the disk was measured. (Figure I)

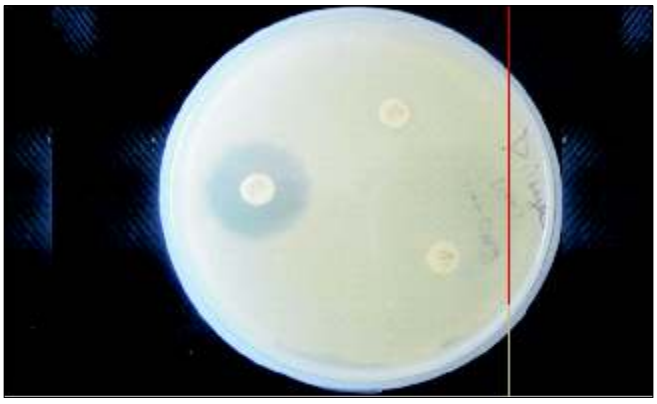

Fig. I. Bacterial isolate depicting susceptibility/resistance towards antibiotics

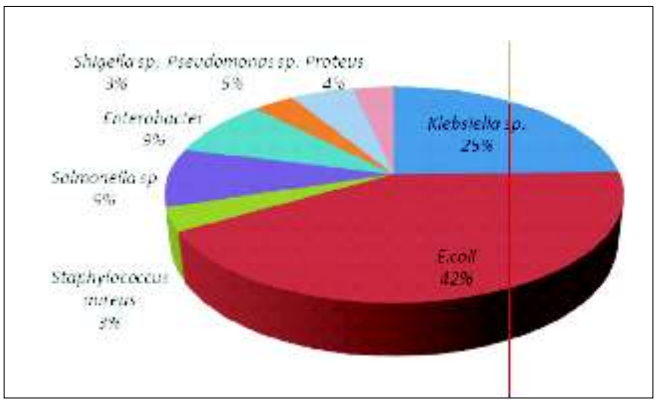

Fig. II. Prevalence of enteric pathogen isolates in ready to eat food samples

RESULTS AND DISCUSSION

Fifty ready to eat food samples *viz.* burger, sandwich, hotdog, pastry, muffin, cream roll, bread, bread cake, chocolate, fruit pudding etc. were used for the isolation of enteric pathogens. Of the 50 ready to eat food samples studied, 57 bacterial strains were isolated. Out of 57 isolates, 24 (42%) isolates were identified as *E. coli*, 14 (25%) isolates were identified as *Klebsiella spp.* Strains belonging to *Salmonella spp.* were isolated from 5 (9%) food samples and *Shigella spp.* was isolated from 2 (3%) food samples. Other isolates viz. *Enterobacter spp., Pseudomonas spp., Proteus spp.* and gram positive organisms were isolated from 5 (9%), 3 (5%), 2 (4%) and 2 (4%) food samples. (Figure II)

The results of this study showed that 14% of the food samples showed the presence of more than one bacterial

species indicating high level of contamination. Serra *et al* [10] had also demonstrated the presence of food borne pathogens (*E.coli, Shigella* and *Salmonella*) from the pastry product in which *E.coli* was the most prevalent pathogen. El-Sukhon[11] showed the presence of *Klebsiella spp.* from 59.7% of raw milk samples. Gastrin et al [12] demonstrated the presence of *Salmonella durham* in cocoa powder used as a confectionary product which caused an outbreak affecting 110 people. *Salmonella spp.* has also been isolated from raw vegetables such as alfalfa sprouts,[13] parsley, cauliflower, lettuce and spinach.[14] Kalantari *et al* [15] demonstrated that sandwich samples (Hamburger, sausage and Kaalbas) were heavily contaminated with *E.coli* (40.3%) followed by *Staphylococcus aureus, Salmonella spp., Klebsiella spp.*

On susceptibility testing it was observed that all bacterial isolates showed resistance to two or more antibiotics as shown in Table 1. The antibiotic susceptibility pattern of *E.coli*, showed that 58 to 91% of the isolates were resistant towards various antibiotics studied. Few studies have shown that it is possible for wild type *E. coli* cultures to acquire spontaneous antibiotic resistance to fluoroquinolones without the loss of any virulence factors. In the present study also *E. coli* isolates showed higher resistance towards fluoroquinolones (58-83%). However *Klebsiella spp.* showed high sensitivity against ciprofloxacin as only 21% of the isolates showed resistance. In contrast earlier studies have reported higher resistance of *Klebsiella spp* towards fluoroquinolones.[16, 17]

In the present study 80% of *Salmonella spp.* isolated were resistant to ciprofloxacin and amoxicillin. All the isolates were resistant to nalidixic acid. Hello *et al* [18] reported worldwide spread of an epidemic population of *Salmonella spp.* and demonstrated that *Salmonella spp.* showed high resistance against ciprofloxacin.

Amongst the gram positive organisms, only two isolates of *S. aureus* were obtained which were resistant to norfloxacin and amoxicillin and one of the isolate was to ciprofloxacin and nalidixic acid also. (Table I)

In the present study, ready to eat food samples were studied for the presence of enteric bacteria. The results of this study showed that *E.coli* was the most prevalent enteric pathogen isolated in the packed food samples. Susceptibility of all isolates to a variety of antimicrobial agents showed high resistance towards the various antibiotics studied. Antibiotic resistance is recognized as a growing problem that poses a major threat to the continued effectiveness of antibiotics used to treat human illness. The prevalence of antimicrobial resistance among food-borne pathogens has increased resulting in increased mortality from bacterial infections that do not respond to common antibiotic therapy.

ACKNOWLEDGMENTS

The study was carried out by the internal grants of the Institute. We are indebted to the Management of Shaheed Udham Singh Group of Institutes for providing the infrastructure and grants required for conducting the study

Table I

Resistance pattern shown by bacterial isolates

	Norfloxacin	Ciprofloxacin	Amoxicillin	Nalidixic acid
E. coli	20 (83%)	(14) 58%	19 (79%)	66% (16)
Klebsiella spp.	7 (50%)	3 (21%)	9 (64%)	12 (85%)
Salmonella spp.	60%	80%	80%	100%
Enterobacter spp.	5 (80%)	2 (40%)	3 (60%)	4 (80%)
Pseudomonas spp.	2 (66%)	1 (33%)	2 (66%)	2 (66%)
Shigella spp.	1 (50%)	0	100%	50%
Staphylococcus aureus	100%	50%	100%	50%
Proteus spp.	1 (50%)	0	100%	50%

Conflict of interest: None

REFERENCES

1. Centers for Disease Control and Prevention. Surveillance for foodborne disease outbreaks-United States, 2007. Morbidity and Mortality Weekly Report 2010;59:973–9.
2. Townes JM, Quick R, Gonzales OY, Linares M, Damiani E, Bopp CA & *et al*. Etiology of bloody diarrhea in Bolivian children: implication for empiric therapy. J Infect Dis 1997;175:1527-30.
3. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States—unspecified agents. Emerg Infect Dis 2011;17:16–22.
4. Bhan MK. Current and future management of childhood diarrhea. Int J Antimicrob Agents 2004;14:71-3.
5. Silbergeld EK, Graham J, Price LB. Industrial food animal production, antimicrobial resistance, and human health. Annu Rev Public Health 2008;29:151-69.
6. Gupta A, Fontana J, Crowe C, Bolstorff B, Stout A, Van Duyne S, *et al*. Emergence of multidrug- resistant *Salmonella enterica* Serotype Newport Infections resistant to expanded-spectrum cephalosporins in the United States. J Infect Dis 2003; 188(11):1707- 16.
7. Little CL, Walsh S, Hucklesby L, Surman-Lee S, Pathak K, Gatty Y, *et al*. Survey of *Salmonella* contamination of non-United Kingdom-produced raw shell eggs on retail sale in the northwest of England and London, 2005 to 2006. J Food Prot 2007;70(10):2259-65.
8. Van TT, Moutafis G, Istivan T, Tran LT, Coloe PJ. *Salmonella*-A dangerous Foodborne Pathogen. Appl Environ Microbiol 2007;73:6885-90.
9. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility testing by a standard single disc method. Am J Clin Pathol 1996;45(4):493-6.
10. Serra MDS, Ferrer EMD, Pericas BE, De S, Serra M, Escobar DF, *et al*. Microbiological quality of pastry products. Anales-de-Bromatologia 1989;41:81-6.
11. El-Sukhon SN. Identification and characterization of *Klebsiella* isolated from milk and milk products in Jordan. Food Microbiol 2003;20:225–30.
12. Gastrin B, Kampe A, Nystrom KG, Oden-Johanson B, Wessel G, Zetterberg B. *Salmonella* durham epidemic caused by contaminated cocoa. Lakartidningen 1972; 69(46)5335-8.
13. Castro-Rosas J, Escartín EF. Incidence and germicide sensitivity of *Salmonella typhi* and *Vibrio cholerae* O1 in alfalfa sprouts. J Food Saf 1999:19(2):137-46.
14. Quiroz-Santiago C, Rodas-Suárez OR, Carlos R V, Fernández FJ, Quiñones-Ramírez EI, Vázquez-Salinas C. Prevalence of *Salmonella* in vegetables from Mexico. J Food Prot 2009;72(6):1279-82.
15. Kalantari S, Sepehri G, Bahrampour A, Sepehri E. Determination of bacterial contamination isolated from Sandwiches in Kerman City and their resistance to commonly used antimicrobials. S Res Lib 2012;4:1100-5.
16. Arlet G, Sanson-le-Pors MJ, Rouveau M, Fourinier G, Marie O, Schlemmer B, *et al*. Outbreak of nosocomial infections due to *Klebsiella pneumonia* producing SHV-4-B-Lactamases: Eur J Clin Microbial Infect Dis 1990;9(11):793-803
17. Meyer KS, Urban C, Eagan JA, Berger BJ, Rahal JJ. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. Ann Intern Med 1993; 119(5):153-8.
18. Le Hello S, Hendriksen RS, Doublet B, Fisher I, Nielsen EM, Whichard JM, *et al*. International Spread of an epidemic Population of *Salmonella enteric* serovar Kentucky ST198 resistant to Ciprofloxacin. J Infect Dis. 2011;204(5):675-84.