Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2404

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **4 (6):** 136-142 (2016)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Antibiotic Resistant *Staphylococcus aureus* from Roadside Sweets and Snacks Sold in Kolkata and Adjoining Regions

Parna Majumder[1], Antara Sarkar[1], Subhasree Maity[1], Chandan Rai[1,2] and Arindam Roy[2*]

[1]Department of Food and Nutrition, RKVM Sarada Ma Girls College, Nabapally, Barasat, 24PGS (N), India

[2]Department of Microbiology, Ramakrishna Mission Vidyamandira, Belur Math, Howrah, India

*Corresponding Author E-mail: arindam_roy97@rediffmail.com

Received: 16.11.2016 | Revised: 27.11.2016 | Accepted: 29.11.2016

ABSTRACT

Food-borne diseases (FBDs) are mostly of short duration and self-limiting but may be fatal for children, aged and debilitating persons. FBDs cause high morbidity and mortality and impose a heavy burden on the gross domestic product. Of the various causes of FBDs, bacterial pathogens have been found to be the commonest. As their route of entry is faecal-oral, proper sewage disposal, self-hygenicity, less bio-aerosols in the air, potable water and over all cleanliness of the food preparation area are important to reduce contamination. Moreover, regular inspection as well as sampling is required to find out the contaminating pathogens and their characteristics. Road-side foods are very popular in Kolkata. These are cheap, nutritious and the choices are plenty. However during summer FBDs are prevalent to the consumers. Of the various bacterial pathogens, Staphylococcus aureus is important producing staphylococcal enterotoxins. This study was undertaken to find out the occurrence and antibiotic resistance pattern of S. aureus in three important road-side sweets and snacks viz., laddu, soan papdi and ghugni.

Key words: *Food-borne diseases, Staphylococcus aureus, Antibiotic resistance, Road-side foods*

INTRODUCTION

Food-borne diseases (FBDs) include food-borne infections caused by contaminating microorganisms growing and colonizing host intestinal tract and food-borne intoxication caused by toxic chemicals already present in food substrate or produced from microbial metabolism. More than 200 pathogens may cause food-borne infection and the microorganisms include viruses, bacteria and protozoa. Viruses are likely to be the most common cause of FBDs but are rarely confirmed as the causative agent because of difficulty in their diagnosis. Bacteria have been found to be the most common cause of FBDs that have been properly investigated and etiologically confirmed. FBDs cause high morbidity and mortality as well as impose heavy burden on the economic development. Food-borne infection is a complex food web involving plants, animals and human beings. Reservoir for food-borne pathogens includes food, water, soil and faeces of humans and other warm blooded animals.

Cite this article: Majumder, P., Sarkar, A., Maity, S., Rai, C. and Roy, A., Antibiotic Resistant *Staphylococcus aureus* from Roadside Sweets and Snacks Sold in Kolkata and Adjoining Regions, *Int. J. Pure App. Biosci.* **4(6):** 136-142 (2016). doi: http://dx.doi.org/10.18782/2320-7051.2404

The pathogenesis of food borne bacterial diseases depends on entry through faecal-oral route, attachment of the pathogens to host tissue, growth and colonization, invasion, evasion of host defense and production of toxin. Most food-borne infections are acute, self limiting and the ailment is of short duration. Though incubation period and duration vary, common symptoms of many FBDs are vomiting, diarrhoea sometimes with blood, abdominal cramp, fever and chills. Most patients recover on their own without medicine but sometimes complication may arise causing dehydration, haemolytic uremic syndrome, irritable bowel syndrome and reactive arthritis[1,2]. Incidence or reason behind FBD is often difficult to evaluate as most cases remain unreported and thus uninvestigated. Many countries have FBD surveillance programme and they maintain the record of FBDs. Unfortunately there is no national data on the prevalence of FBDs in India and thus the predominant food-borne pathogens and their behaviour remain ill-defined.

Of the various food-borne bacterial pathogens, staphylococci are important causing staphylococcal food poisoning. Staphylococci are facultative anaerobic gram positive spherical bacteria producing grape like cluster of cells due to cell division in more than one plane. Staphylococcal cell wall is resistant to lysozyme but sensitive to lysostaphin. Carotenoids present in their cells protect them from oxidizing substances[3]. Many members of the genus *Staphylococcus* predominantly *S. aureus* and *S. epidermidis* are associated as permanent or transient flora and often colonize skin surface, sweat and sebaceous glands, mucous membrane of nose and pharynx of healthy human beings. Thus foods are easily contaminated by staphylococcal carriers during food processing or post-preparation handling. Moreover growth at wide range of temperature (range 7°-48.5°C; optimum 30°-37°C), pH (range 4.2-9.3; optimum 7-7.5), sodium chloride (up to 15%) and at low water activity gives ample scope of metabolism to the contaminating staphylococci[4]. Coagulation of blood is a characteristic feature of *S. aureus* differentiating them from other staphylococci collectively designated as coagulase negative staphylococci[5.] The virulence factor of staphylococcal food poisoning called staphylococcal enterotoxin (SE) is protein secreted into the food medium after production within the cytoplasm. More than 14 types of SEs have been documented which are water soluble and resistant to denaturation and thus causing severe outbreak. Different types of SEs are produced by strains of both coagulase positive and coagulase negative staphylococci isolated from foods. The type of foods that have been mostly associated with staphylococcal food poisoning varies greatly from one country to another depending on their food habit and method of preparation. However, the common foods causing staphylococcal outbreak include milk and dairy products including cheese, cream, butter, meat and poultry products, canned food, eggs, salads etc.

Kolkata is famous for its road-side culinary. Laddu and soan papdi are two popular sweets made principally from Bengal gram flour. Ghugni is another popular snack of road-side eateries. Contact with hands during moulding and serving of laddu and soan papdi is very common. Ghugni is also easily contaminated during open-air cooking, uncovered storing and garnishing. As these processes may contaminate these foods with bacteria including *S. aureus* and storage of these foods may favour their growth and metabolism, these foods were chosen to study the presence of *S. aureus* and their antibiotic resistance pattern

MATERIALS AND METHODS

Collection of sample

Three different types of samples viz. ghugni, laddu and soan papdi totaling twenty-seven were purchased from road side shops selling snacks and sweet in and around Kolkata. Samples were collected aseptically in sterile container and brought to the laboratory within 2h of collection for analysis in an ice box.

Measurement of pH

Sample was mixed with distilled water, blended to make a paste and pH measured with a digital pH meter.

Presumptive isolation of *S. aureus*

Ten gram of representative food sample was homogenized with 90ml sterile peptone-physiological saline (0.1% w v^{-1} neutral peptone, 0.85% w v^{-1} sodium chloride, pH 7.2) by shaking in an orbital rotary shaker at 150 revolutions per min for 2min. Ten-fold Serial dilutions were prepared using the same diluent. 0.1 ml of appropriate dilutions was spread-plated on sterile dried plates containing *Baird Parker* agar base supplemented with egg yolk emulsion and potassium tellurite solution (BPA) and the plates were incubated for 24h at 35°C. Grey black shiny colonies surrounded by clear zone having 1-1.5 mm diameter after 24h were regarded as *S. aureus* (Figure 1.).At least three representative colonies were randomly selected from each positive sample and purified by repeated streaking on freshly prepared BPA plates. Purified colonies were grown on nutrient agar slants and stored at 4°C.

Confirmation of *S. aureus*

Presumptive isolates were confirmed as *S. aureus* by positive mannitol fermentation and DNase production.

Mannitol fermentation

Mannitol fermentation was tested on mannitol salt agar plate by line streak inoculation of 18h old cultures grown in nutrient agar and incubating the plates at 35°C for 24h. Production of yellow or white colonies surrounded by yellow zone indicated mannitol fermentation.

Thermostable DNase production test

Production of DNase was tested on DNase test agar with methyl green as indicator. 18h old culture supernatant was heat treated at 100°C for 10 min and then was spot inoculated and the inoculated plates were incubated at 35°C for 24h. Production of clear zone indicated thermostable DNase production.

Antibiotic sensitivity pattern

Antibiotic sensitivity pattern of the thirty-six isolates was tested by disc agar diffusion method against twelve antibiotics. Three colonies, grown on tryptone soya agar at 37°C for 24h, were transferred to about 5ml tryptone soya broth and incubated at the same temperature for 4-6h until the broth became moderately turbid. A sterile cotton swab was dipped into the inoculum and applied evenly onto Mueller-Hinton agar plate (4mm thick). After drying for 15min, various antibiotic susceptibility test discs were applied aseptically keeping a distance of at least 3cm between their centers. The plates were incubated at 35°C for 18h. The zones showing complete inhibition were measured and designated as sensitive, intermediate or resistant based on manufacturer's zone-size interpretative chart.

RESULT AND DISCUSSION

Altogether twenty-seven numbers of three different types of foods categorized as sweets and snacks were purchased from roadside shops in and around Kolkata. All these foods were subjected to analysis for pH, standard plate count, *B. cereus* and *S. aureus*. However, only *S. aureus* is being reported here. *S. aureus* is present in fourteen numbers i.e., 51.8% of samples (Table 1.). Food-wise analysis shows that most of the ghugni samples were contaminated. *S. aureus* count ranged from a maximum of 6.37 log cfu g^{-1} present in laddu to a minimum of 5.32 log cfu g^{-1} in ghugni. Average *S. aureus* count was also highest in laddu (6.23 log cfu g^{-1}**)** and lowest in ghugni (5.91 log cfu g^{-1}) samples. In another study 80% of the laddu samples tested from Bangalore city was found to be contaminated with *S. aureus*. 88% of these sixteen isolates were coagulase positive[6].

Fig. 1: BPA plate showing typical *S. aureus* colonies

Table 1: *S. aureus* in samples studied

Parameter	Ghugni	Laddu	Soan papdi	Total
Samples studied	9	10	8	27
Positive sample	5	5	4	14
% positive sample	55.6	50	50	51.85
Range (log cfu g^{-1})	6.21-5.32	6.37-5.97	6.29-5.69	6.37-5.32
Average (log cfu g^{-1})	5.91	6.23	6.07	6.19

Increasing air pollution, demand pressure, scarcity of potable water, lack of hygienic practices and temperature abuse increase the chances of contamination. The source of *S. aureus* may be food handlers as the same person handle money and serve sweets with bare hands[7,8]. Food processing where hands are used for moulding balls of laddu, addition of water to cooked ghugni, mixing of previous lot with fresh lot of ghugni and storage uncovered are other suspected sources of staphylococcal contamination.

The staphylococci are ubiquitous in nature with humans and animals as the primary reservoirs. They are found in air, dust, water, as well as human and animal wastes[9]. The threshold level for staphylococcal food poisoning is around 10^5 cfu staphylococci g^{-1} of food producing sufficient amount of enterotoxin (20ng – 1µg) to produce the typical symptoms[10]. Incubation period of staphylococcal food poisoning ranges from 2-6h and the typical symptoms include nausea, vomiting, abdominal cramp, retching and prostration. In more severe cases muscle cramp, sudden fall in blood pressure and pulse rate may also occur.

Coagulase production and growth and acid production on mannitol salt agar medium are accepted as characteristic features of pathogenic staphylococci[11]. A total of thirty-six isolates were confirmed as *S. aureus* from the three types of foods analyzed. These isolates were tested for antibiotic resistance pattern against twelve antibiotics (Table 2.). Of the twelve isolates from ghugni samples all were resistant to amoxyclav, ampicillin, methicillin and penicillin G. At least seventy-five percent of these isolates were sensitive to chloramphenicol, ciprofloxacin, norfloxacin and polymixin B. All the isolates from laddu samples were resistant to amoxyclav,

ampicillin and methicillin. More than seventy-five percent of these isolates were resistant to penicillin G and trimethoprim also. All the isolates from soanpapdi were resistant to amoxyclav, ampicillin and penicillin G while sensitive to chloramphenicol, ciprofloxacin and norfloxacin. More than ninety percent of thee isolates were sensitive to tetracycline and polymixin B. Overall *S. aureus* isolates from these three types of foods showed resistance towards β-lactam antibiotics and trimethoprim. Sensitivity was higher towards protein synthesis, nucleic acid synthesis and cell membrane synthesis inhibitors.

Table 2: Antibiotic resistance pattern of the *S. aureus* isolates

Antibiotic (disc^{-1})	Ghugni (n=12)			Laddu (n=13)			Soan papdi (n=11)			Total
	S	I	R	S	I	R	S	I	R	R %
Cell wall synthesis inhibitor										
Amoxyclav (30µg)			12			13			11	100
Ampicillin (10µg)			12			13			11	100
Methicillin (5µg)			12			13	4		7	89
Penicillin G (10U)			12	1		12			11	97
Vancomycin (30µg)	8	1	3	5	1	7	6	4	1	31
Protein synthesis inhibitor										
Chloramphenicol (30µg)	10		2	8		5	11			19
Erythromycin (15µg)	5	3	4	5	1	7	7	1	3	39
Tetracyclin (30µg)	8	2	2	7		6	10	1		22
Nucleic acid synthesis inhibitor										
Ciprofloxacin (5µg)	11		1	7		6	11			19
Norfloxacin (10µg)	9		3	6		7	11			28
Cell membrane synthesis inhibitor										
Polymixin B (300U)	10		2	7		6	10		1	25
Folic acid synthesis inhibitor										
Trimethoprim (5µg)	3		9	2		11	8		3	64

Higher resistance towards ampicillin, methicillin and penicillin G and sensitivity towards chloramphenicol, ciprofloxacin and erythromycin is in accordance to other researchers[6]. Our finding of 31% vancomycin resistance is also supported by them where 41% of the coagulase positive *S. aureus* were vancomycin resistant. This is very alarming as vancomycin is the drug of last resort against many gram positive bacterial pathogens. However, 100% vancomycin sensitivity has been reported by others[12,13].

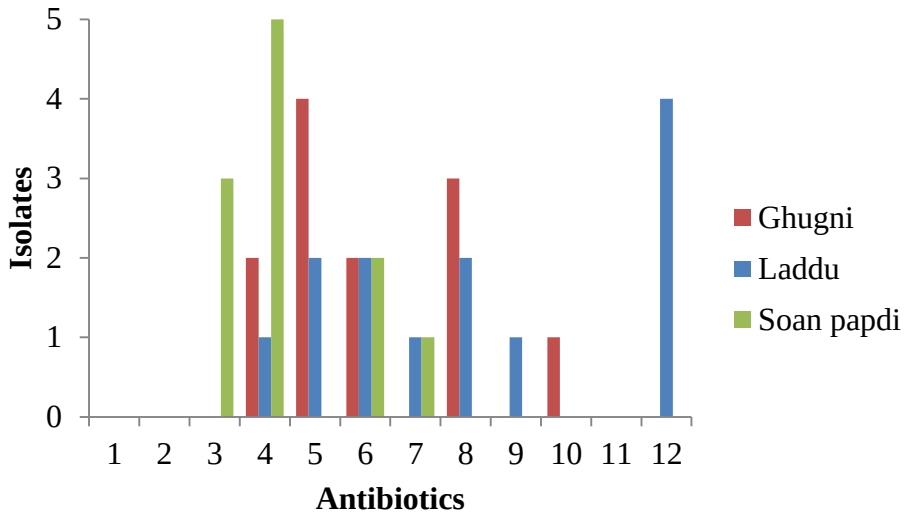

Fig. 2: *S. aureus* isolates resistant against number of antibiotics

Figure 2. shows the number of isolates that are multiple-drug-resistant. All the isolates from these types of foods were multiple-drug resistant (MDR) with number of antibiotics showing resistance ranging from three to twelve. Of the thirteen laddu isolates, drug resistance ranged from a minimum of four to a maximum of twelve antibiotics. More than thirty percent laddu isolates were resistant against twelve antibiotics. Similarly range of drugs against which ghugni and soan papdi isolates showed resistance were from four to ten and three to seven antibiotics, respectively.

Antibiotic resistance is a global issue and is compared to the problem of global warming in its spectra and consequence. Antibiotic abuse takes place at various points by the patients as well as medical practitioners. Irrational and indiscriminate prescription even of carbapenems and sulbactams by medical practitioners as well as self medication, over the counter purchase and incomplete course of antibiotic uptake by patients resulted in increasing number of multiple-drug-resistant strains. Pathologists should also be cautious enough in reporting culture and in-vitro sensitivity assay as doctor's prescription is guided by their reports. So both the stake-holders viz. doctors and patient should judiciously use this wonder drug to overcome possible future menace. Finally Government should implement effective antibiotic policy based on antibiotic resistance data and prescription audit[14].

CONCLUSION

Fifty percent of the three popular sweet and snack foods viz. laddu, soan papdi and ghugni sold in road-side eateries in and around Kolkata were found to be contaminated by *S. aureus*. Mis-handling, prolonged storage at ambient temperature and mixing of left-over ghugni to the fresh stock may favour metabolism of the pathogens to produce staphylococcal enterotoxin in these types of foods. This may cause staphylococcal food poisoning to the consumers that may be fatal to the children, old and the debilitated persons. Antibiotic sensitivity assay reveals that all the pathogens were MDR and many were resistant to methicillin and vancomycin. β-lactam drugs were least effective while protein synthesis and nucleic acid synthesis inhibitor drugs were most effective.

Acknowledgement

Authors are thankful to Ramakrishna Vivekananda Mission Sarada Ma Girls College authority for providing financial and infrastructural assistance and Principal, Ramakrishna Mission Vidyamandira for providing infrastructural facility and financial assistance through Swami Vivekananda Research Centre (SVRC).

REFERENCES

1. Spiller, R., Aziz, Q. and Creed, F., Guidelines on the irritable bowel syndrome: mechanisms and practical management. *Gut,* **56(12):** 1770–1798 (2007).
2. Kadariya, J., Smith, T.C. and Thapaliya, D., *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health, *Biomed Res Int*. **2014:** 1-9 (2014).
3. Liu, G.Y., Essex, A., Buchanan, J.T., Datta, V., Hoffman, HM., Bastian, J.F. et al., *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J Exp Med*, **202:** 209–215(2005).
4. Loir, Y.L., Baron, F. and Gautier, M. *Staphylococcus aureus* and Food poisoning, *Genet Mol Res*, **2:** 63-76 (2003).
5. Ryan, K.J. and Ray, C.G. Sherris Medical Microbiology: An Introduction to Infectious Diseases, 4th edn. McGraw Hill Publishers (2004).
6. Subbaiah, U.M. and Subramanyam, V., Comparison of antibiotic resistance pattern of coagulase positive and coagulase negative *Staphylococcus aureus* isolates from Indian sweets, *Int J Res Pharm Sci*, **2(4):** 38-47 (2012).
7. Kasturwar, N.B. and Shafee, Md., Knowledge, Practices and Prevalence of MRSA among Food Handlers, *Int J Biol Me Res*, **2(4):** 889-894 (2011).
8. Ray, S.K., Dobe, M., Lahiri, A. and Basu, S.S. Hand washing practices in urban and rural communities in and around Kolkata, West Bengal, *Indian J Public Health*, **53(3):** 192-5 (2009).
9. Bergdoll, M. and Lee, W.A., Staphylococcal intoxication. Foodborne Infections and Intoxications; Reimann, P. and Cliver, D. 3rd Edition; Academic Press, Elsevier, New York, USA, pp 523-525 (2006).
10. Salandra, G., Goffredo, E., Pedarra, C., Nardella, M., Parisi, A., et al., Occurrence, characterization and antimicrobial resistance pattern of *Staphylococcus* species isolated from dairy products in southern Italy, *Int J Food Microbiol*, **9:** 327-360 (2008).
11. Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S. and Fitzpatrick, E.S., Veterinary Microbiology and Microbial Disease, 2nd Edition, Wiley-Blackwell (2011).
12. Sina, H., Baba-Moussa, F., Kayode, A.P., Noumavo, P.A., Sezan, A., Hounhouigan, J.D., Kotchoni, S.O., Prevost, G. and Baba-Moussa, L. Characterization of *Staphylococcus aureus* isolated from street foods: Toxin profile and prevalence of antibiotic resistance, *J Appl Biosci.*, **46:** 3133-3143 (2011).
13. Ghosh, S. and Banerjee, M., Methicillin resitance and inducible clindamycin resistance in *Staphylococcus aureus*, *Indian J Med Res*, **143(3):** 362-364 (2016).
14. Bhattacharya, P.K., Emergence of antibiotic-resistant bacterial strains, methicillin-resistant *Staphylococcus aureus*, extended spectrum beta lactamases, and multi-drug resistance is a problem similar to global warming, *Revista da Sociedade Brasileira de Medicina Tropical*, **47(6):** 815-816 (2014).