The Internet Journal of Microbiology
Volume 7 Number 1

Homologous Resistant Groups (HRGs) in Multidrug Resistant Staphylococcus aureus Isolated from Retail Foods in Vadapalani, Chennai

M Manoharan, S Jayaraman, S Illanchezian, J Jayasekar

Citation

M Manoharan, S Jayaraman, S Illanchezian, J Jayasekar. *Homologous Resistant Groups (HRGs) in Multidrug Resistant Staphylococcus aureus Isolated from Retail Foods in Vadapalani, Chennai*. The Internet Journal of Microbiology. 2008 Volume 7 Number 1.

Abstract

The present study investigated the prevalence of multiple antibiotic resistant Staphylococcus aureus strains in different food sources collected from retail shops and markets in Vadapalani, Chennai. All S. aureus isolates were resistant to methicillin, and 93.75 % of them were multidrug resistant. The S. aureus isolates were typed by UPGMA method based on antibiotic resistance pattern observed. Approximately five homologous resistance groups (HRGs) were observed in typing the isolates.

INTRODUCTION

Food borne diseases are of major concern worldwide. To date, around 250 different food-borne diseases have been described and bacteria are the causative agents for two-thirds of the food-borne disease outbreaks. Among the predominant bacteria involved in these diseases, Staphylococcus aureus is the leading cause of gastroenteritis resulting from consumption of contaminated food (Loir et al., 2003). Occasionally, S. aureus acts as opportunistic pathogen and cause infections of the urinary tract, respiratory tract and intestinal tract (Coltman, 1981). The pathogenicity of S. aureus as indicated by Stokes and Ridgway (1980) include abscesses, boils, conjunctivitis especially in newborn, cross-infections in hospitals septicemia, mastitis and food poisoning (of meats, milk and milk products). Also S. aureus is an etiological infection agent responsible for significant levels of morbidity and mortality (Vieira et al., 2007).

S. aureus is an important pathogen due to the combination of toxin-mediated virulence, invasiveness and antibiotic resistance (Fast et al., 1989; Bohach et al., 1990; Hiramatsu, 1995; Murray et al., 1995). The evolution of methicillin resistant Staphylococcus aureus (MRSA) and other drug resistant pathogens has been linked to extensive antibiotic use in medicine and food animal production (Leonard and Markey, 2007; Mathew et al., 2007). Outbreaks of Staphylococcal food poisoning (SFP) are very common across the world; however, there is only one report on SFP from the Indian subcontinent (Nema et al., 2007). The aim of this study was to investigate the prevalence and multiple antibiotic resistance of Staphylococcus aureus strains isolated from different food samples collected from retail shops and markets in Vadapalani, Chennai and also to type the isolates based on antibiotic resistance pattern. This study brings insight into the predominance of multiple drug resistant S. aureus in marketed food samples in Chennai.

MATERIALS AND METHODS

BACTERIAL ISOLATION

A total of 40 different food samples collected from retail shops and markets in Vadapalani, Chennai were subjected to S. aureus isolation. All the samples were aseptically handled and processed. The samples were plated onto Mannitol Salt Agar (HiMedia). The characteristic yellow colored, round colonies were isolated and stored on Nutrient Agar (HiMedia) slants. The isolates were examined by Gram staining for Gram positive cocci in clusters and biochemical characteristics such as catalase positive, oxidase negative, indole negative, urease positive, citrate negative, Methyl red and Voges-Proskauer positive. All isolates were confirmed as S. aureus by their positive nature for tube coagulase test using rabbit plasma. The standard type strain S. aureus MTCC 1144 was used in each of the steps for the comparative identification of S. aureus.

ANTIBIOTIC ASSAY

Antibiotic resistance test was carried out for all the strains by the disc diffusion method (Bauer et al., 1966). The cultures were enriched in Brain Heart Infusion Broth (BHIB, HiMedia) for 6 - 8 hr. The enriched cultures were then swabbed on the surface of Mueller Hinton Agar (HiMedia) using sterile cotton swabs. Using the antibiotic disc dispenser, the discs were placed on the agar surface sufficiently distanced to avoid overlapping of the inhibition zones. The concentration of each antibiotics used is listed in Table 1. The plates were incubated at 37 $^{\circ}$ C for 18 - 24 hr. After the incubation period, the diameter of the inhibition zones were measured and compared with the performance standards for antimicrobial disc susceptibility tests (Zone size interpretative chart, HiMedia) and classified accordingly as resistant, intermediate and sensitive.

MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDEXING OF THE ISOLATES

The MAR index of a single isolate is defined as a / b, where 'a' represents the number of antibiotics to which the isolate was resistant and 'b' represents the total number of antibiotics to which the isolate was subjected (Krumperman, 1985).

TYPING OF S. AUREUS ISOLATES

All the S. aureus isolates were typed based on their similarity index in antibiotic resistance pattern. Similarity index (S.I.) was calculated by Dice's coefficient as,

Figure 1

$$S.I. = \frac{2 * N_{AB}}{(N_A + N_B)}$$

Where N_{AB} is number of similar antibiotics resistant to both strains; N_A is the total number of antibiotics resistant to strain 1; and N_B is the total number of antibiotics resistant to strain 2.

Cluster analysis was performed using the unweighted pair-group method with average linkages (UPGMA, Sneath and Sokal, 1973). The isolates showing similar antibiotic resistance pattern and S.I. value of 1 were grouped as homologous resistance group (HRG).

RESULTS

INCIDENCE

About 80% (32 / 40) of the food samples collected from retail shops and markets in Vadapalani, Chennai were positive for contamination with S. aureus. Of the strains isolated, 50% was observed in seafood, 18.75% in fruits and 12.5% in packaged milk samples.

ANTIBIOTIC RESISTANCE

All the S. aureus isolates were resistant to methicillin. More than 80% of the strains were resistant to ampicillin (93.75%), novobiocin (93.75%) and amoxyclav (84.38%). The isolates showed intermediate resistance towards bacitracin (62.5%) and kanamycin (59.38%). About 31.25% of the isolates were resistant to vancomycin. All the isolates were sensitive to gentamicin and norfloxacin (Table 1).

Figure 2

Table 1 Percentage of antibiotic resistance of isolates from food samples

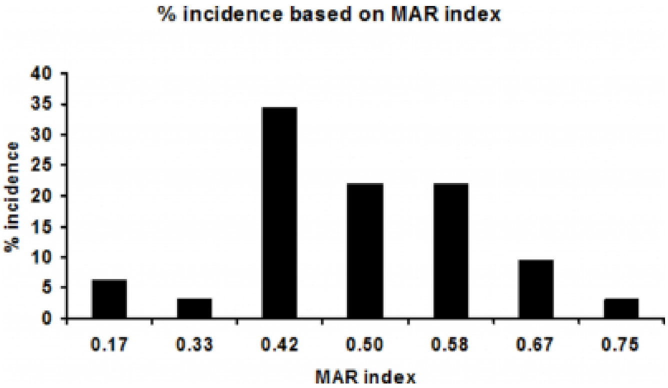
Antibiotics	Concentration	No. of Strains (n= 32)	% resistance
Methicillin (M)	5mcg	32	100
Ampicillin (A)	30mcg	30	93.75
Novobiocin (Nv)	30mcg	30	93.75
Amoxyclav (Am)	30mcg	27	84.38
Bacitracin (B)	10units	20	62.5
Kanamycin (K)	30mcg	19	59.38
Vancomycin (V)	30mcg	10	31.25
Nitrofurantoin (Nf)	300mcg	10	31.25
Erythromycin (E)	15mcg	8	25
Tetracycline (T)	30mcg	3	9.38
Gentamicin (G)	10mcg	0	0
Norfloxacin (Nx)	10mcg	0	0

MAR

About 93.75% of the S. aureus isolates showed multiple antibiotic resistance. The MAR index value was in the range of 0.17 – 0.75. Only 2 strains did not show multiple antibiotic resistance. One of the S. aureus isolate showed resistance to 9 of the 12 antibiotics tested. Notably, 34.38% of the isolates had a MAR index value of 0.42 (Fig. 1).

Figure 3

Fig. 1 Percentage occurrence of MAR index of isolates from food samples (n=32)



ANTIBIOTIC RESISTANCE TYPING

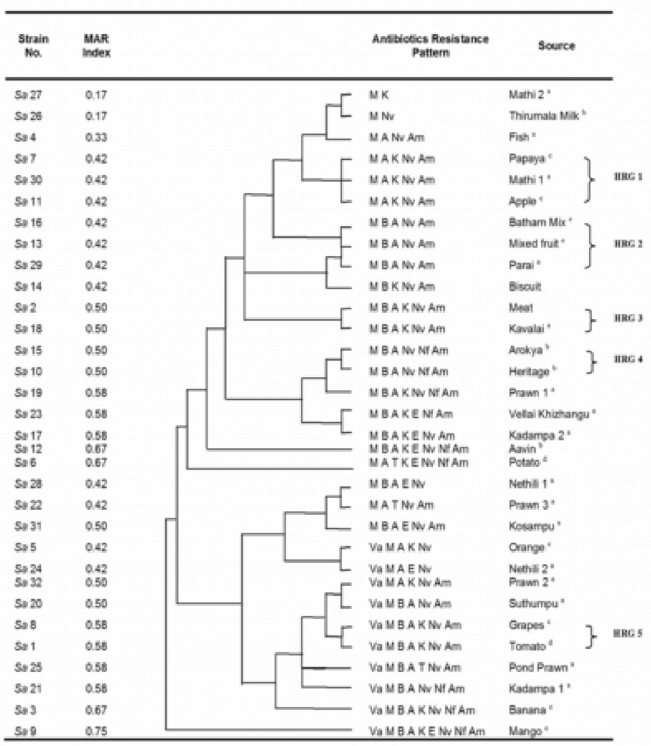
All the S. aureus isolates were typed based on their antibiotic resistance pattern. Approximately 5 HRGs (homologous resistance groups) were observed in the present study. Notably, 4 / 5 HRG consist of S. aureus isolated from similar sources. Only 1 HRG was observed in the isolates resistant to vancomycin (Fig. 2).

DISCUSSION

This is the first report on the prevalence of multiple antibiotic resistant S. aureus isolated from marketed food samples in Vadapalani, Chennai. About 80% incidence of S. aureus

Figure 4

Fig. 2 Typing of isolates from food samples based on antibiotics resistance pattern



HRG – Homologous Resistance Group

[a] seafood samples collected from Vadapalani; [b] milk samples collected from Vadapalani; [c] fruit samples collected from Vadapalani; [d] vegetables collected from Vadapalani; [e] juice samples collected from Vadapalani

was observed in the food samples taken for analysis. These results are high compared to that documented by other researchers (Genigeorgis 1989; Sokari 1991; Wieneke et al., 1993; Haeghebaert et al., 2002; Aycicek et al., 2005; Chomvarin et al., 2006). Similar to the previous studies, all S. aureus isolates were methicillin resistant (MRSA) (Kluytmans et al., 1995; Leonard and Markey 2007). Prevalence of vancomycin resistant S. aureus (VRSA) was also observed in the food samples. Most of the isolates were resistant to ampicillin and novobiocin and all the isolates were sensitive to gentamicin and norfloxacin. The multiple antibiotic resistance (MAR) index value observed in this study was high.

In the present study, the wide spread contamination of MRSA in food samples was observed. All the isolates were sensitive to gentamicin and norfloxacin. This is because of the less (or) nil exposure of food samples to these antibiotics, whereas, some clinical isolates have shown resistance to gentamicin and norfloxacin (Daxboeck et al., 2004; Harbarth et al., 2005; Farzana and Hameed 2006). Notably, vancomycin resistance was observed in some of the fishes and fruits only. The use of antibiotics against pests and microbes is the main reason for increased resistance to antibiotics. Some have reported the plasmid borne resistance of S. aureus against antibiotics (Best et al., 1977; Amyes et al., 1987; Bhakta et al., 2004). Thus spreading of the strains harboring resistance plasmids and natural transformation of these plasmids to non-resistant strains would pose a threat during treatment of diseases.

The incidence of S. aureus and their antibiotic resistance in different food samples have been documented (Barreto and Vieira 2003; Ekici et al., 2004; Normanno et al., 2007). There have been reports on food initiated disease outbreak of S. aureus (Kluytmans et al., 1995; Jones et al., 2002; Jadhav et al., 2007). Some researchers have reported that food handlers act as carriers of antibiotic resistant S. aureus (Wei and Chiou 2002; Vieira et al., 2007; Ingham et al., 2007).

A novel approach of typing S. aureus based on similarity index calculated upon antibiotic resistance pattern was described in the present study. The similarity index between the isolates was in the range of 0.22 to 1.0. The antibiotic resistance pattern observed in the homologous resistance groups are HRG 1 (M A K Nv Am), HRG 2 (M B A Nv Am), HRG 3 (M B A K Nv Am), HRG 4 (M B A Nv Nf Am) and HRG 5 (Va M B A K Nv Am). About 4 HRG groups, 2 each in MAR index value of 0.42 and 0.5 respectively was observed. The other HRG was observed in the MAR index value of 0.58. It was observed that, as the MAR index increases, the occurrence of HRG decreased. The source of the S. aureus isolates in 4/5 HRG was similar: HRG 1 & HRG 5 (fruits), HRG 2 (juices) and HRG 4 (milk), except that of HRG 3 which contained unrelated sources. This method of typing is useful in describing the effective chemotherapeutic treatments in case of disease outbreaks with multiple antibiotic resistant Staphylococcus aureus.

ACKNOWLEDGEMENTS

We thank the management of Life Teck Research Centre, Chennai for providing us the facilities and requisite support for completion of this work. We thank all our co-workers for providing us constant support throughout this work and also in the successful preparation of this manuscript.

References

r-0. Amyes SGB, Young HK, Skurray RA (1987) Plasmid-mediated rimethoprim-resistance in Staphylococcus aureus. Biochem J 243:309–312

r-1. Aycicek H, Cakiroglu S, Stevenson TH (2005) Incidence of Staphylococcus aureus in ready-to-eat meals from military cafeterias in Ankara, Turkey. Food Control 16(6):531–534

r-2. Barreto NSE, Vieira RHSF (2003) Investigação sobre possíveis portadores de Staphylococcus aureus em duas indústrias de pesca. Arquivo de Ciências, Impressa Universitária da UFC 17:49–57 (Português)

r-3. Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standard single disc method. Am J Clin Pathol 36:493–496

r-4. Best GK, Wood DO, Carter MJ (1977) Plasmid-mediated resistance to gentamicin in Staphylococcus aureus. Antimicrob Agents Chemother 12(4):513–517

r-5. Bhakta M, Arora S, Bal M (2003) Intraspecies transfer of a chloramphenicol-resistance plasmid of staphylococcal origin. Indian J Med Res 117:146–151

r-6. Bohach GA, Fast DJ, Nelson RD, Schlievert PM (1990) Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit Rev Microbiol 17:251–272

r-7. Chomvarin C, Chantarasuk Y, Srigulbutr S, Chareonsudjai S, Chaicumpar K (2006) Enteropathogenic bacteria and enterotoxin-producing Staphylococcus aureus isolated from ready-to-eat foods in Khon Kaen, Thailand. Southeast Asian J Trop Med Public Health 37(5):983–990

r-8. Coltman K (1981) Urinary tract infections. New thoughts on an old subject. Pract 223:351–355

r-9. Daxboeck F, Assadian O, Apfalter P, Koller W (2004) Resistance rates of Staphylococcus aureus in relation to patient status and type of specimen. J Antimicrob Chemother 54:163–167

r-10. Ekici K, Bozkurt H, Isleyici O (2004) Isolation of some pathogens from raw milk of different milch animals. Pak J Nutr 3(3):161–162

r-11. Farzana K, Hameed A (2006) Resistance pattern of clinical isolates of Staphylococcus aureus against five groups of antibiotics. J res (Sci), Bahauddin Zakariya University, Multan, Pakistan 17(1):19–26

r-12. Fast DJ, Schlievert PM, Nelson RD (1989) Toxic shock syndrome-associated staphylococcal and streptococcal pyrogenic toxins are potent inducers of tumor necrosis factor production. Infect Immun 57:291–294

r-13. Genigeorgis CA (1989) Present state of knowledge on staphylococcal intoxication. Int J Food Microbiol 9:327–360

r-14. Haeghebaert S, Le Querrec F, Gallay A, Bouvet P, Gomez M, Vaillant V (2002) Les toxi-infections alimentaires collectives en France, en 1999 et 2000. Bull Epidémiol Hebdo 23:105–109 (French)

r-15. Harbarth S, François P, Schrenzel J, Rodriguez CF, Hugonnet S, Koessler T, Huyghe A, Pittet D (2005) Community-associated methicillin-resistant Staphylococcus aureus, Switzerland. Emerg Infect Dis 11(6):962–965

r-16. Hiramatsu K (1995) Molecular evolution of MRSA. Microbiol Immunol 39:531–543

r-17. Ingham SC, DeVita MD, Wadhera RK, Theis ML (2007) Assessing the potential of Streptococcus pyogenes and Staphylococcus aureus transfer to foods and customers via a survey of hands, hand-contact surfaces and food-contact surfaces at foodservice facilities. J Foodservice 18(2):76–79

r-18. Jadhav SL Lt Col, Sinha Surg AK Lt Cdr, Banerjee A Col, Chawla PS Lt Col (2007) An outbreak of food poisoning in a military establishment. Med J Armed Forces India 63:130–133

r-19. Jones T, Kellum M, Porter S, Schaffner W (2002) An outbreak of community-acquired foodborne illness caused by methicillin-resistant Staphylococcus aureus. Emerg Infect Dis 8(1):82–84
r-20. Kluytmans J, Van Leeuwen W, Goessens W, Hollis R, Messer S, Herwaldt L, Bruining H, Heck M, Rost J, Van Leeuwen N, Van Belkum A, Verbrugh H (1995) Food-initiated outbreak of methicillin-resistant Staphylococcus aureus analyzed by pheno- and genotyping. J Clin Microbiol 33(5):1121–1128
r-21. Krumperman PH (1985) Multiple antibiotic indexing of E. coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol 46:165–170
r-22. Leonard F, Markey B (2007) Methicillin-resistant Staphylococcus aureus in animals: a review. Vet J. Doi:10.1016/j.fvjl.2006.11.008
r-23. Loir YL, Baron F, Gautier M (2003) Staphylococcus aureus and food poisoning. Genet Mol Res 2(1):63–76
r-24. Mathew AG, Cissell R, Liamthong S (2007) Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog Dis 4(2):115–133
r-25. Murray DL, Ohlendorf DH, Schlievert PM (1995) Staphylococcal and streptococcal superantigens: their role in human diseases. ASM News 61:229–235
r-26. Nema V, Agrawal R, Kamboj DV, Goel AK, Singh L (2007) Isolation and characterization of heat resistant enterotoxigenic Staphylococcus aureus from a food poisoning outbreak in Indian subcontinent. Int J Food Microbiol 117(1):29–35
r-27. Normanno G, Corrente M, La Salandra G, Dambrosio A, Quaglia NC, Parisi A, Greco G, Bellacicco AL, Virgilio S, Celano GV (2007) Methicillin-resistant Staphylococcus aureus (MRSA) in foods of animal origin produced in Italy. Int J Food Microbiol 117(2):219–222
r-28. Sneath PHA, Sokal R (1973) Numerical taxonomy: the principles and practices of classification. WH Freeman and Co., San Francisco, California, USA.
r-29. Sokari T (1991) Distribution of enterotoxigenic Staphylococcus aureus in ready-to-eat foods in eastern Nigeria. Int J Food Microbiol 12(2–3):275–279
r-30. Stokes JE, Ridgway GL (1980) Clinical Bacteriology. Arnold E (ed), 5th edn. Sevenoaks, UK, pp 35–50
r-31. Vieira RHSF, Albuquerque WF, Macrae A, Sousa OV, Vieira GHF (2007) Multiple drug resistant Staphylococcus aureus strains isolated from a fish market and from fish handlers. Braz J Microbiol 38:131–134
r-32. Wei HL, Chiou CS (2002) Molecular subtyping of Staphylococcus aureus from an outbreak associated with a food handler. Epidemiol Infect 128:15–20
r-33. Wieneke AA, Roberts D, Gilbert RJ (1993) Staphylococcal food poisoning in the United Kingdom, 1969-1990. Epidemiol Infect 110:519–531

Author Information

Muthu Saravanan Manoharan, B.Tech.
Department of Biotechnology, Life Teck Research Centre

Sathishkumar Jayaraman, B.Tech.
Department of Biotechnology, Life Teck Research Centre

Seethalakshmi Illanchezian, M.Phil.
Department of Biotechnology, Life Teck Research Centre

Jasmine Jayasekar, M.Phil.
Department of Biotechnology, Life Teck Research Centre