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Prevalence of Antimicrobial Resistance in *Staphylococcus aureus* Isolated from Ready to Eat Foods, Hand Swabs and Utensil Swabs of Street Vendors Selling Food on Wheels

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A B S T R A C T

The prevalence of antibiotic resistance of *Staphylococcus aureus* in 50 samples of ready to eat food, hand swabs and utensil swabs of street vendors was studied. 58% of samples were contaminated with *S. aureus*. The highest count ($5.66x10^4$cfu/$25cm^2$) was found in hand swab samples. The antibiotic resistance of the isolates was tested using 12 antibiotics. 72.41% isolates were resistant to Erythromycin while 31.03% isolates were resistant to Ampicillin, Ofloxacin and Sparfloxacin. Overall 62% of *S. aureus* isolates were multidrug resistant. The study indicated that ready to eat food samples, utensils used in their preparation and vendors serving hands are frequently contaminated with *S. aureus* and their resistance pattern can cause greater risk in transfer of resistance to other bacteria through food chain. This could be common route for spread of resistant *Staphylococcus* food borne infections.

Introduction

Staphylococcal food-borne disease (SFD) is one of the most common food-borne diseases worldwide resulting from the contamination of food by preformed *S. aureus* enterotoxins. However, several studies have documented prevalence of *S. aureus* in many food products including raw retail meat indicating that consumers are at potential risk of *S. aureus* colonization and subsequent infection. *S. aureus* is a commensal and opportunistic pathogen that can cause wide spectrum of infections, from superficial skin infections to severe, and potentially fatal, invasive disease (Lowy, 1998). This ubiquitous bacterium is an important pathogen due to combination of "toxin-mediated virulence, invasiveness and antibiotic resistance." This organism has emerged as a major pathogen for both nosocomial and community-acquired infections. *S. aureus* can cause contamination of food products during food preparation and processing. *S. aureus* can grow in a wide range of temperatures (7° to 48.5° C; optimum 30 to 37°C), pH (4.2 to 9.3; optimum 7 to 7.5), and sodium chloride concentration up to 15% NaCl. *S. aureus* is a desiccation tolerant organism with the ability to survive in potentially dry and stressful environments, such as the human nose and on skin and inanimate surfaces such as clothing and surfaces (Chaibenjawong and Foster, 2011). These characteristics favour growth of

the organism in many food products (le Loir, Baron, 2003). *S. aureus* can remain viable on hands and environmental surfaces for extended durations after initial contact (Kusumaningrum *et al.,* 1990).

The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades. There is a growing tendency through the consumption of fast foods (hot or cold ready-to-eat foods), which increases the risk of food-borne diseases.

Improper food handling practices in the retail food industry are thought to contribute to a high number of FBD outbreaks (Lues, 2007). It was reported that the hands of food handlers were implicated in 42% of food-borne outbreaks that occurred between 1975 and 1998 in the United States (Ayçiçek *et al.,* 2004.). In a recent study (Syne *et al.,* 2013) investigated the microbiological contamination in ready-to-eat food products processed at a large processing plant in Trinidad, West Indies, *S. aureus* was the most common pathogen detected. The overall prevalence of *S. aureus* detected in air, food, and environmental samples was 27.1% (46/170). It was determined that the counts of *S. aureus* increased after heat treatment, and only post cooking environmental surfaces that came into contact with ready-to-eat foods that were contaminated with *S. aureus* during slicing and packaging harboured *S. aureus*. The hands of ready-to-eat food service employees have been shown to be vectors in the spread of food borne disease, mainly because of poor personal hygiene. Investigations of food borne illness outbreaks have shown that poor personal hygiene, primarily ineffective hand washing, is an important contributor to food borne illness, secondly to inadequate temperature controls of food (Scarborough, 2002). A number of data confirm that *S. aureus* cause many outbreaks of food poisoning resulting from hand contact (Bryant *et al*., 1988). Antimicrobial resistance associated with food and water has been a global concern (Kumar *et al*.,). It is now widely accepted that there is an association between the use of antimicrobial agents and the occurrence of resistance. Antimicrobials exert a selective pressure on microorganisms that act as driving force in the development of antibiotic resistance. Moreover, there remains the possibility that resistance may be transmitted from antibiotic resistant bacteria to the susceptible ones (Kessie *et al.,* 1998). Multidrug resistant bacteria in foods threaten the efficacy of human drugs if antimicrobial resistance genes become incorporated into bacterial population (Smith *et al*., 2002).

So the objective of this study was to investigate the prevalence of food-borne *S. aureus* in ready to eat food sold by street vendors, hand swabs and utensils swabs of food handlers and their susceptibility to commonly used antimicrobials. This will help to determine the potential hazards and public health implications that may be closely concerned with consumption of these foods prepared by street vendors.

Materials and Methods

Sample description

Ten samples of each ready to eat food (Vegetables burger, Vegetables momos, Noodles), hand swabs and utensil swabs of street vendors were taken. 100gm of each solid sample was taken with sterile forceps in sterile plastic zipper bag. Hand swab and utensil swab samples of food handlers were taken using sterile metal grid of 25cm^2 and sterile cotton swabs dipped in maximum recovery diluents. The swabs were then transferred to diluent tube. All the samples were transported to laboratory and analysed within 1 hour of collection or refrigerated at 4°C before being analysed.

Sample analysis

10gm of sample was taken in sterile stomacher bag containing 90ml of sterilized maximum recovery diluent (Hi-media).The sample was homogenised for 30 seconds using a stomacher to prepare uniform suspension. Serial dilutions of all samples were prepared in maximum recovery diluents (Hi-media). From each dilution tube, 0.1 ml was spread on to each of the two agar plates (Blood agar and Baird Parker agar) in duplicate. The blood agar plates were incubated at 37°C overnight and Baird Parker agar plates at 37°C for 30 hrs. After incubation, typical colonies of *S. aureus* (Shiny black convex colonies with or without narrow grey white margins on Baird Parker agar and golden yellow coloured colonies on blood agar plates) were retained for further confirmation. Negative and positive control was also kept to compare the growth of colonies. The total number of viable colonies (cfu/gram) of sample was also determined (IS 5887-2).

Characterization of isolates

Isolates were confirmed as being *S. aureus* by the coagulase test (both slide and tube coagulase test). Further confirmation was done by catalase activity, growth in nutrient agar supplemented with 7.5% and 10% Nacl, nitrate reduction and Gram's staining.

Antimicrobial susceptibility testing

The susceptibility of *Staph aureus* isolates to antimicrobial agents was tested by disc diffusion method (Kirby- Bauer technique (Bauer *et al*., 1966) using Muller-Hinton agar and antibiotic discs (Hi-media) as per guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2003. Performance standards for antimicrobial Disc Susceptibility tests, 8th ed. Approved standard M2-A8, NCCLS, Wayne, PA, USA.). Twelve antibiotics discs of Amikacin (AMK), Ampicillin (AMP), Amoxycillin (AMX), Azithromycin (AZ), Ciprofloxacin (CIP), Erythromycin (ERY), Clindamycin (CL), Tetracycline (TET), Gentamycin (GEN), Oxycillin (OXY), Ofloxacin (OFL) and Sparfloxacin (SPR) were used to test antibiotic sensitivity of *Staphylococcus aureus* isolates. The antibiotic discs were placed on the agar plates previously inoculated with 18 hrs broth cultures of isolated test organisms. The plates were incubated at 37^{0}C for 24 hrs. The size of the inhibition zones exhibited by test organisms against different antibiotics was measured. Results on the basis of zone diameter were recorded as sensitive, intermediate or resistant to a particular antibiotic with according to the NCCLS recommendations. *Staphylococcus aureus* MTCC 3160 was used as reference strain.

MIC determination

MIC determination was done as per guidelines of NCCLS, 2003(NCCLS, 2003. Methods for Dilution Antimicrobial Susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M7-A6, NCCLS, Wayne, PA, USA.).

The cell suspensions were prepared using overnight growth of fresh culture of selected strains to give inoculum size according to 0.5 McFarland standards to determine MIC. The appropriate inoculum size used for standard MIC was 2 x10^6 CFU/ml. The working dilutions of antibiotics of different concentration used were prepared from stock solution. Using the multipipettor, 100 µl of medium was dispensed into all wells of a microtitre plate. The plate and lid was labelled. 100 µl of appropriate 2x antibiotic solutions was pipette into the wells in column 1 (far left of plate) of microtitre plate. Using

the multipipettor set at 100 µl mixed the antibiotics into the wells in column 1 by sucking up and down 6-8 times. Then 100µl from column 1was withdrawn and added this to column 2. This made column 2 a twofold dilution of column 1. It was mixed by sucking up and down 6-8 times. It was then transferred 100 µl of column 2 to column 3. The procedure was repeated down to column 10 only. 100 µl from column 10 was discarded rather than putting it in column 11. Thus column 11 was control without antibiotic. Inoculum with set inoculums size 2 x 10^6cfu/ml was poured into a sterile petri dish. With the smaller multipipettor set at 5 µl, the wells of the microtitre plate were inoculated with 5 µl of inoculum starting from columns 11 to 1 in that order. Inoculum was not added to column 12 (sterility control and blank for the plate scanner). Microtitre plates were incubated at 37°C for 24 hours. The purity of the bacterial culture was checked by streaking the bacterial cultures on Mueller-Hinton agar plates. When satisfactory growth in the form of turbidity was obtained after incubation of 18-36 hours, the microtitre plate was scanned for optical density (O.D) at 620nm using an ELISA reader (TECAN).Using column 12 as the blank, MIC was taken as the lowest concentration of drug that reduces growth by more than 50% or 90% for MIC_{50} or MIC_{90} respectively.

Results and Discussions

Hands of ready-to-eat food serving vendors have been shown to involved in the spread of food borne pathogens mainly because of poor personal hygiene and which may account for approximately 97% of food borne illnesses in food service establishments and homes. Out of 50 samples taken for analysis, 58% of samples were contaminated with *Staphylococcus aureus.* In Table 1, the highest *S. aureus* count in the hand swab samples ($5.66x10^4$cfu/ $25cm^2$) and utensil swab sample ($2.628x10^3$cfu/$25cm^2$) indicated that the food in question has been exposed to condition that might allow the spread of spread of pathogens. Food sample such as Vegetable Burger, Vegetable Momos and Noodles were also contaminated but highest count among food samples was in noodles ($3.5x10^2$cfu/gm). Although count from food samples taken was less than that of hand swab samples but it may increase with poor holding and handling of these ready to eat samples.

Staphylococcus aureus is an important food poisoning organism because of its cosmopolitan distribution in nature. Temperature of 30-37^{0}C at which samples were collected favoured the growth of this organism. Outbreaks of Staphylococcus food poisoning have been reported to occur as the result of contamination of precooked food, often through unsanitary handling and holding food at temperature that allow the growth and toxin production (Newsome, 1988; Bergdoll, 1989; Syndor and Poland, 1991).

The microbial contamination of ready to eat foods could be closely related to preparing method and its handling. The sanitary condition of the environment under which these are being sold may also lead to their contamination. Highest count of staphylococcus in hand swab indicated that unwashed hands can transmit pathogens to food products during serving and dirty serving utensils may contribute to increase the count. Most of the food vendors lack proper education and adequate knowledge on how best food could be handled without contamination. Unsold product left are usually presented for sale the next day perhaps with gentle heat treatment could also be hazardous as some strains of *Staphylococcus aureus* have elevated thermal resistance, which cause inactivation by current culinary heating technique.

Table.1 Average count of *Staphylococcus aureus* in ready to eat food and swab samples

Sample	No of samples taken	Percentage of samples contaminated	Average cfu/gm or cfu/ 25cm^2
Vegetable Burger	10	50	2.2x10^{2}cfu/gm
Vegetable Momos	10	30	1.3x10^{2}cfu/gm
Noodles	10	10	3.5x10^{2}cfu/gm
Hand swab	10	100	5.66x10^{4}cfu/ 25cm^2
Utensil swab	10	100	2.628x10^{3}cfu/ 25cm^2
Total	**50**	**58**	

Table.2 Antibiotic Resistance in *Staphylococcus aureus* strains isolated from ready to eat food and swab samples

Name of the Sample	No. of *S.aureus* isolates tested	Percentage Resistance to antibiotics											
		Amk	Amp	Amx	Az	Cipro	Ery	Cld	Tet	Gent	Oxy	Ofl	Spr
Vegetable Burger	5	60	20	60	60	60	100	60	40	60	60	40	40
Vegetable Momos	3	67	67	67	67	67	67	67	67	67	67	67	67
Noodles	1	0	0	100	0	100	100	0	100	100	0	0	100
Hand swab	10	60	20	40	20	30	60	30	40	20	40	20	40
Utensil swab	10	30	40	40	30	40	60	20	40	30	40	30	20
Total	**29**	**48.27**	**31.03**	**48.27**	**34.48**	**44.82**	**72.41**	**34.48**	**44.82**	**37.93**	**44.82**	**31.03**	**31.03**

Table.3 Multidrug resistance of *Staphylococcus aureus* isolates of different samples to selected antibiotics

Sample	No of *S.aureus* isolates tested	Number resistant to 2 or more antibiotics	Occurrence (%)
Vegetable Burger	5	5	100
Vegetable Momos	3	2	66
Noodles	1	1	100
Hand swab	10	5	50
Utensil swab	10	5	50
Total	**29**	**18**	**62**

Table.4 MIC (µg/ml) determination of antibiotics in *Staphylococcus aureus* isolates of different samples

Antibiotics	***Staphylococcus aureus* isolates of different samples**				
	SaVB	SaVM	SaN	SaHS	SaUS
AMK	**32**	**64**	**32**	**32**	**32**
AMP	**32**	**64**	**32**	**32**	**32**
AMX	**32**	**16**	**16**	**16**	**32**
AZ	**1**	**2**	**1**	**1**	**2**
CIP	**8**	**8**	**16**	**4**	**8**
ERY	**8**	**16**	**8**	**8**	**8**
CL	**8**	**8**	**16**	**8**	**8**
TET	**32**	**32**	**16**	**16**	**16**
GEN	**32**	**16**	**16**	**16**	**16**
OXY	**32**	**31**	**32**	**32**	**64**
OFL	**8**	**16**	**16**	**8**	**8**
SPR	**2**	**1**	**1**	**2**	**1**

***SaVB, SaVM, SaN, SaHS, SaUS** (*Staphylococcus aureus* isolates of Vegetables Burger, Vegetables Momos, Noodles, Hand swab and Utensil swab)

The antibiotic sensitivity test with selected 12 antibiotics was conducted to detect the multidrug resistance among isolates of *Staphylococcus aureus*. Most of the isolated strains exhibited antibiotic resistance indicates that the ready to eat food products could also poses a public health risk to consumers.

Table-2 summarizes the resistance pattern of all the *S. aureus* strains to 12 antibiotics. The higher percentage (72.41%) of the isolates was resistant to erythromycin while lower percentage (31.03 %) of isolates was resistant to Ampicillin, Ofloxacin and Sparfloxacin. Most of the strains resistant for Erythromycin were isolated from Vegetable Burger and Noodles samples. High resistance was also found (48.27%) for Amikacin and Amoxicillin. Table-3 indicated that 62% of *S. aureus* strains were multidrug resistant (Resistance to two or more antibiotics). Although food and swab samples were obtained from street vendors at different locations and at different times, the isolates showed similar resistance pattern.

MIC of selected 12 antibiotics against only one isolate of *Staphylococcus aureus* of each category of sample was determined. Table 4 summarizes the MIC determination of selected antibiotics in *Staphylococcus aureus* isolates of different samples. The MIC (µg/ml) ranged between 1-64µg/ml. Highest MIC 64(µg/ml) of Amikacin and Ampicillin was observed in *Staphylococcus aureus* isolates of Vegetables Momos and of Oxycillin in isolates of utensil swab. Lowest MIC (1µg/ml) of Sparfloxacin was observed in *Staphylococcus aureus* isolates of Vegetable Momos, Noodles and utensil swab and MIC (1µg/ml) of Azithromycin was observed in *Staphylococcus aureus* isolates of Vegetable Burger, Noodles and Hand swab samples.

The present study indicated that consumption of ready to eat foods contaminated with multidrug resistant strains of *Staphylococcus aureus* is fatal. This study also revealed that hand hygiene and utensil cleanliness was also unsatisfactory and may cause serious implications for public health due to

contamination of food from food handlers' hands and their serving utensils. The food handlers need adequate training on both personal and environmental hygiene to prevent poor preparation, distribution and serving of food. There is great need to educate the street vendors especially in India selling ready to eat cheap foods on wheels about hazards of *Staphylococcus aureus* contamination. Control measures such as displaying foods in closed glass cabinets, washing hands at regular intervals, not serving food with bare hands and selling off all food items on the same day should be adopted to prevent food infections from occurring and developing in society. The antibiotic resistance pattern of isolates of *Staphylococcus aureus* in ready to eat food samples observed in this study suggest a greater risk in transfer of resistance to other bacteria because they can form commensal flora through food chain. The level of resistance to antimicrobial drug is a reflection of the indiscriminate misuse and abuse of antibiotics in the environment (Umoh *et al.*, 1990; Chigbu and Ezeronye, 2003). A regular surveillance is required to detect the presence and detection of multidrug resistant contaminants in ready to eat food and their selling environment.

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