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Prevalence and Antibiotics Resistance Patterns of *Staphylococcus aureus* Isolated from Kitchen Sponge's at Jimma Town Food Establishments, South West Ethiopia

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Abstract: *A cross-sectional study was carried out to investigate the prevalence and antibiotic resistance patterns of Staphylococcus aureus isolated from kitchen sponges used in food establishments of Jimma town, between October, 2010 and June, 2011. A total of 201 kitchen sponge samples from 20 restaurants, 101 hotels, 47 cafeterias and 33 pastry shops were enrolled in this study. Antibiotics susceptibility patterns of S. aureus isolates were done using 12 selected antibiotics. Out of 201 samples examined 69(34.3%) kitchen sponges were found to have S. aureus. Isolation rates of S. aureus differed among the food establishment types and it ranged from 30% (restaurants) to 36.4% (hotels). Significant variation in prevalence of S. aureus among kitchen sponges of restaurants, hotels, pastry shops and cafeterias were revealed (p=0.034). Ampicillin and Streptomycin were the most resisted drugs. Norfloxacin, Amikacin and Ciprofloxacin showed maximum sensitivity. Nine (9) drug resistance patterns were detected among S. aureus isolates. There was significant variation in the prevalence of Staphylococcus aureus. Kitchen sponges used in food service establishments of Jimma town* recognized as potential agents in the spread of microorganisms, *and the isolates showed high resistant patterns to Ampicillin and Streptomycin.*

Keywords: *S. aureus, Prevalence, Antibiotic Resistance, Kitchen Sponges, Jimma Town*

1. INTRODUCTION

It is known that during the cleaning process of equipment, utensils, etc. in kitchens, the pre-washing and washing steps are done with the use of sponges to eliminate food residues. As a consequence of this procedure, part of the food residues adheres to the sponge surfaces. These food residues together with the moisture retained in the sponges offer a favorable environment for microbial growth. Early studies on bacterial contamination in the kitchen were conducted in the late 1960s investigating bacterial load of hand towels and the hygienic conditions of domestic dishcloths and tea towels. Such cloths were heavily contaminated with bacteria and suspected as one of the main vectors for spreading and dissemination of the bacteria in the kitchen [1]. The current attention on bacterial contamination in the kitchen was started in the late 1970s. Previous studies have suggested that although raw material is probably the main source of contamination in the kitchen, the area surrounding the kitchen could also act as sources of free living populations of bacteria. Sponges and dishcloths have been recognized as potential agents in the spread of microorganisms, it has been observed that bacteria persist in these vehicles [2].

The preamble of the Codex Alimentarius Commission states that adequate, safe, sound and wholesome food is a vital element for the achievement of acceptable standards of living and that the right to a standard of living adequate for the health and wellbeing of the individual and his family is proclaimed in the Universal Declaration of Human Rights of the United Nations. Routine food establishments' inspection and control is a responsibility of the Ethiopian Health and Food Regulatory

Authority. Therefore, it is probable that there is a risk of *S. aureus* poisoning in humans through food served at food establishments in Jimma town.

Common sources of *S. aureus* are Man's respiratory passages, skin and superficial wounds. When *S. aureus* is allowed to grow in foods, it can produce a toxin that causes illness. Although cooking destroys the bacteria, the toxin produced is heat stable and may not be destroyed. Staphylococcal food poisoning occurs most often in foods that require hand preparation, such as potato salad, ham salad and sandwich spreads. Sometimes these types of foods are left at room temperature for long periods of time, allowing the bacteria to grow and produce toxin. Good personal hygiene while handling foods will help keep *S. aureus* out of foods, and refrigeration of raw and cooked foods will prevent the growth of these bacteria if any are present [3].These observations with variations in the sensitivity patterns reported for *S. aureus* stress the significance of continuous monitoring of antibiotic sensitivity patterns to provide suitable guidelines for treatment. Ethiopia is a socio-economically deprived region where both personal and community hygiene are minimal. According to records of public and private hospitals, enteric fever is major infectious disease occurring at high fluctuating incidences.

The rationale of conducting the research in Jimma town is due to the fact that Jimma town is one of the main gates to south western part of Ethiopia and middle point between Addis Ababa and south western Ethiopia. It has lot of natural places for visiting and trade port for both sides that are to central Ethiopia and south western part of the country. Therefore, every year many individuals from different parts of the country travel to and away from the city. In addition, numbers of food venders are increasing in the town. It became an important and urgent matter to evaluate safety of kitchen sponges; no literatures have ever been found in Ethiopia concerning microbiological safety of kitchen sponges of food establishments. Moreover, there are no reports on current antibiotic sensitivity patterns of *S.aureus* isolates from kitchen sponges in this region; this study assessed the multidrug resistance among *S. aureus* isolates.

Hence, the result of this study would be used to develop food sanitation strategy in the town, to achieve the target of government policy and to have better life quality for all individuals, and would provide Information on the antimicrobial resistance pattern of the *S. aureus* isolates from kitchen sponges for successful treatment, as well as planning strategic use of drugs to minimize the incidence of resistance bacteria in the future.

2. MATERIALS AND METHODS

2.1. Study Area and Study Period

The study was conducted from October, 2010 to June, 2011 in Jimma town. According to Jimma town Central Statistics Office, the town has a population size of 127,945 and located 353 kilometers southwest of Addis Ababa. Laboratory activities were carried out at Postgraduate and Research Laboratory of College of Natural Science, Jimma University.

2.2. Study Design and Sampling

A cross-sectional study design was formulated to determine the prevalence and antibiotic resistance patterns of *S.aureus* isolated from Kitchen Sponges at Jimma town food establishments. A total of 423 food establishments that were identified by the town trade and small scale enterprise office were used as the sampling frame. The establishments were stratified by the type of service they provide into the following strata: 101 hotels, 20 restaurants, 47 cafeterias and 33 pastry shops. Purpose of stratification was to avoid over or under representation of certain types of establishments. A proportional sample size was determined for each stratum. Two hundred one (201) synthetic sponges involved in daily use in households were collected from randomly selected different food establishments in Jimma town. Sponges collected from food establishments were transported in a sterile polyten bags to avoid contamination. Samples were transported to the Laboratory and analyzed within 1-3hrs.

2.3. Isolation of *Staphylococcus aureus*

Mannitol Salt Agar (Oxoid), and Nutrient Broth (Oxoid) supplemented with 7.5 %(w/v) sodium chloride were used for culturing of samples. The sterility of these media was checked by incubating

the media overnight before its use. Yellow Colonies from Mannitol Salt Agar (MSA) were picked and inoculated into Nutrient Broth containing 7.5% sodium chloride and MSA according to [4]. In brief, the inoculums from the Nutrient Broth were inoculated on pre-sterilized and surface-dried media by streaking aseptically to obtain discrete colonies. The plates were incubated at 37^0C for 24-36hrs under aerobic conditions and left at room temperature for pigment formation. After incubation, the culture plates were examined recording to the appearance, size, colour, and morphology of the colonies. The characteristic colonies (yellow mannitol fermenting colonies on MSA plates) were aseptically picked, further purified by repeated streaking and characterized using established microbiological methods that include colonial morphology, cell shape and grouping, Gram reaction, catalase and coagulase tests.

Purified colonies were tested for catalase production by removing several colonies of the test organism using a sterile wooden stick or a glass rod and immersed into 3% hydrogen peroxide solution. Production of bubbles of oxygen was an indication for catalase production. Slide coagulase test were done in order to identify *S. aureus*. In slide test (detects bound coagulase), a colony of the purified isolates were emulsified in a drop of distilled water on two separate slides to make two thick suspensions. A loopful of plasma were added to one of the suspensions and mixed gently. Clumping within 10seconds was observed if the organism was a coagulase producer. Isolates that were Gram-positive, coccus shaped catalase positive and capable of coagulating human plasma was considered as *S. aureus*.

2.4. Antimicrobial Susceptibility Testing for *Staphylococcus aureus*

The antimicrobial susceptibility patterns of the isolates were determined according to Kirby Bauer disc diffusion technique as described by National Committee for Clinical Laboratory Standard [4, 5]. The following 12 drugs were used to determine the antibiogram of the isolates: Penicillin G (6µg), Erythromycin (15µg), Ampicillin (10µg), Amikacin (30µg), Chloramphenicol (30µg), Gentamycin (10µg), Streptomycin (10µg), Kanamycin (30µg), Methicillin (10µg), Ciprofloxacin (5µg), Tetracycline (25µg) and Norfloxacin (10µg). Standardized (McFarland 0.5 or barium sulphate turbidity standard equivalent of $3x10^8$cfu/ml cell density) in sterile nutrient broth suspension of the test organism was used to swab the surface of Mueller Hinton agar plates and dried while the Petri dish lid is in place for no longer than 15minutes at ambient temperature [6]. A set of 6 standard antimicrobial discs were placed on the inoculated Mueller Hinton agar plates using sterile forceps and allowed to dry at room temperature. Then after, Plates were incubated at 35^0C for 16-18hrs. The diameters of the zone of inhibition produced by each antimicrobial disc were measured, recorded and the isolates were interpreted as resistant, intermediate or susceptible based on the standard [4, 5]. Standard reference strains, which included *Staphylococcus aureus* (ATCC 25923) was used as quality control of the discs used. The reference strains were kindly obtained from Ethiopian Health and Nutrition Research Institute (EHNRI), Ethiopia.

McFarland 0.5 turbidity standards were prepared as per the standard guidelines described by the Clinical and Laboratory Standards Institute (CLSI). Before each use, the standards were shaken well, mixing the fine white precipitate of barium sulfate in the tube. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer.

2.5. Data Management and Analysis

Data entry and analysis were done using the Statistical Package for Social Sciences (SPSS, version 16.0). Both descriptive and analytical statistical methods were applied. Frequency and percentages were computed to describe the relevant variables. P-value of 0.05 was taken as cut-off for statistical significance.

2.6. Ethical Clearance

Ethical clearance was obtained from Research and Ethical Clearance Committee of Natural Sciences, Jimma University and Jimma Health Bureau as well. Data at the food establishments were collected with full consent of head/or owners of the establishments. The study objectives were clearly explained to the food establishment owners and each head of the establishment was assured that the information provided would be kept confidential and used only for the purpose of the research.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Isolation Rates of S.aureus

From a total of 201 samples examined 69 (34.3%) kitchen sponges from food establishments were found to be positive for *Staphylococcus aureus* (Table 1). Moreover, the isolation rates of *S. aureus* was 6(30%) from kitchen sponges of restaurants, 36 (36.4%) kitchen sponges of hotels, 11 (33.3%) of kitchen sponges of pastry shops and 16 (34%) of cafeteria's kitchen sponges. The isolation rates of *S. aureus* differed among the food establishment types and it ranged from 30% (restaurant) to 36.4% (hotels) (Table1). The statistical analysis revealed that presence of significant variation in prevalence of *S. aureus* among kitchen sponges of restaurants, hotels, pastry shops and cafeterias (p= 0.034).

3.1.2. Antimicrobial Susceptibility Patterns of Staphylococcus aureus

A total of 69 isolates were tested against twelve (12) commonly used antimicrobials viz. including Penicillin G (6µg), Erythromycin (15µg), Ampicillin (10µg), Amikacin (30µg), Chloramphenicol (30µg), Gentamycin (10µg), Streptomycin (10µg), Kanamycin (30µg), Methicillin (10µg), Ciprofloxacin (5µg), Tetracycline (25µg) and Norfloxacin (10µg) following NCCLS 2000 guidelines. Among all the antimicrobials tested, Streptomycin (100%) and Ampicillin (100%) were the most resisted drugs followed by Penicillin G (97.1%), Methicillin (92.7%), Kanamycin (76.8%), Chloramphenicol (72.5%), Tetracycline (20.3%) and Gentamycin (8.7%). Norfloxacin (0%), Amikacin (0%) and Ciprofloxacin (0%) showed maximum sensitivity (Table 2).

3.1.3. Multi Drug Resistance Patterns of S. aureus

A total of 7 multi drug resistance (MDR) patterns were detected among *Staphylococcus aureus* isolates. Out of the 69 isolates only 1(1.4%) was resistant to 3 antibiotics, 4(5.8%) of the isolates were resistant to 4 of the antibiotics tested, 18(26%) were resistant to 5 antibiotics, 27 (39.1%) were resistant to 6 antibiotics, 13 (19%) were resistant to 7 antibiotics, whereas 5 (7.3%) were resistant to 8 antibiotics, only 1(1.4%) isolate was resistant to 9 antibiotics (Table 3).

Table1. *Prevalence of S. aureus from kitchen sponges of restaurants, hotels, pastry shops and cafeterias (n=201).*

Food establishment types	Sample size	*S. aureus* positive	% *S. aureus* positive	P value
Restaurant	20	6	30	p=0.034
Hotels	101	36	36.4	
Pastry shops	33	11	33.3	
Cafeteria	47	16	34	
Total	201	69	34.3	

Table2. *Antimicrobial resistance of S. aureus isolates of kitchen sponges by food establishment types (n=69)*

Antimicrobial disc	Total *S. aureus* (n=69) isolate	Total number (%) isolates resistant from kitchen sponges of			
		Restaurant n=6(%)	Hotel n=36(%)	Cafeterias n=16(%)	Pastry shop n=11(%)
Tetracycline	14(20.3)	1(16.7)	8(22.2)	3(18.8)	2(18.2)
Streptomycin	69(100)	6(100)	36(100)	16(100)	11(100)
Penicillin G	67(97.1)	6(100)	35(97.2)	15(93.8)	11(100)
Norfloxacin	0(0)	0(0)	0(0)	0(0)	0(0)
Methicillin	64(92.7)	6(100)	33(91.7)	15(93.8)	10(90.9)
Kanamycin	53(76.8)	5(83.3)	28(77.8)	12(75)	8(72.7)
Gentamycin	6(8.7)	0(0)	4(11.1)	1(6.25)	1(9.1)
Erythromycin	20(29)	2(33.3)	10(27.8)	5(31.3)	3(27.3)
Ciprofloxacin	0(0)	0(0)	0(0)	0(0)	0(0)
Chloramphenicol	50(72.5)	4(66.7)	26(72.2)	12(75)	8(72.7)
Ampicillin	69(100)	6(100)	36(100)	16(100)	11(100)
Amikacin	0(0)	0(0)	0(0)	0(0)	0(0)

Table3. *Multidrug resistance pattern of S. aureus isolated from kitchen sponges of food establishments of Jimma town.*

MDR patters	Resistance patters	Number of isolates	Percent (%)
Three	Str,Kan,Amp	1	1.4
Four	Pen,Str,Kan,Amp	2	2.9
	Pen,Str,Amp,Met	2	2.9
Five	Pen,Str,Kan,Amp,Met	10	14.5
	Ery,Str,Kan,Amp,Tet	1	1.4
	Pen,Str,Amp,Met,Chl	5	7.3
	Pen,Str,Gen,Amp,Met	1	1.4
	Pen,Str,Kan,Amp,Chl	1	1.4
Six	Pen,Str,Kan,Amp,Met,Chl	22	31.9
	Pen,Ery,Str,Amp,Met,Chl	2	2.9
	Pen,Str,Gen,Amp,Met,Chl	1	1.4
	Pen,Ery,Str,Amp,Met,Chl	2	2.9
Seven	Pen,Str,Kan,Amp,Met,Tet,Chl	2	2.9
	Pen,Ery,Str,Kan,Amp,Met,Chl	3	4.4
	Pen,Ery,Str,Amp,Met,Tet,Chl	4	5.9
	Pen,Ery,Str,Kan,Amp,Met,Chl	3	4.4
	Pen,Ery,Str,Kan,Amp,Met,Tet	1	1.4
Eight	Pen,Ery,Str,Kan,Amp,Met,Tet,Chl	4	5.9
	Pen,Ery,Str,Kan,Gen,Amp,Met,Chl	1	1.4
Nine	Pen,Ery,Str,Kan,Gen,Amp,Met,Chl,Tet	1	1.4

Where AMP= Ampicillin, STR= Streptomycin, NOR= Norfloxacin, TET= Tetracycline, KAN= Kanamycin, GEN= Gentamycin, CHL= Chloramphenicol, CIP= Ciprofloxacin, AMK= Amikacin, ERY=Erythromycin, MET= Methicillin, PEN= Penicillin G.

3.2. Discussion

This was the first time report on Prevalence and Antibiotics Resistance Patterns of *Staphylococcus aureus* isolated from Kitchen Sponge's at Jimma town Food Establishments, South West Ethiopia. Hence, the results of this study are discussed, compared and contrasted with similar and related studies in other countries.

Outbreaks of food poisoning frequently occur as a result of improper food preparation in which cross-contamination in combination within inadequate storage or cooking was implicated in many instances [7]. Dishcloths and sponges were recognized as a potential source for spreading microorganisms and it was observed that bacteria persisted in these vehicles [8]. From the results of this study, about 34.3% of the kitchen sponges of food establishments of Jimma town were found to have *Staphylococcus aureus*.

With regard to the prevalence of *S. aureus* from kitchen sponge's sampled from the four food establishment types indicated that there was significant difference. Relatively, high prevalence was obtained in samples from hotels and cafeterias. This might be indicating the prolonged usage of kitchen sponge when compared to other food establishment types. In fact, the hygiene and sanitary conditions of the kitchen among pastry shops during sample collection were better than kitchen of other food establishment types. In other work reported that the prevalence of *S. aureus* isolates were 20% from kitchen sponges and 19% from dishcloths [9].But, the present study revealed about 34.3% *S. aureus* among kitchen sponges of food establishments. This may be due to the poor hygienic conditions that are being practiced in food establishments of Jimma. Staphylococci are the normal flora of many meat animals and they are also part of the normal flora of man, residing in nasal passage, throat and skin (Baired–Parker, 1974) cited by [10]. Because of this ubiquitous occurrence of the bacteria in nature, they are often found in food processing materials [11]. *Staphylococcus epidermidis, Staphylococcus aureus* causes infections from use of foreign materials like catheters and prosthesis. Though it is a normal flora of the skin and mucous membranes and was regarded as a contaminant, and invasion of this organism may cause severe infection and sometimes can be very fatal [12]. The presence and growth of *S. aureus* in food along its enterotoxin is a potential public health hazard [13]. One of the major factors contributing to staphylococcal food poisoning outbreaks is humans' carriers, who handle food in food service areas, homes, and food processing plants [14].

The symptoms of staphylococcal intoxication are produced when a toxin dose of less than 1.0μg is present in the contaminated food. However, in immune-compromised people, a dose of 100-200ng is sufficient to cause illness [15]. *Staphylococcus aureus* can be found on clothing and utensils handled by humans. Out of the 201 samples examined 34.3% kitchen sponges from the four food establishments' types were positive for *Staphylococcus aureus*. Moreover that, *S. aureus* were isolated from 30% kitchen sponges of restaurant, 36.4% kitchen sponges of hotels, 33.3% of kitchen sponges of pastry shops and 34% of cafeterias kitchen sponges. The isolation rates of *S. aureus* differed among the food establishment types and it ranged from 30% (restaurant) to 36.4% (hotels). The statistical analysis revealed that presence of significant variation in prevalence of *S. aureus* among kitchen sponges of restaurant, hotels, pastry shops and cafeterias. The variation in prevalence of *S.aureus* among the food establishment types is may be due to difference on duration of use of sponges to wash utensils.

Antimicrobial susceptibility study of *S. aureus* isolates by disc-diffusion method indicated that the isolates were highly resistant to the commonly used antibiotics in the country. For the antimicrobial resistance of the *S aureus* isolated from kitchen sponges of the town food establishments, the high resistance levels were observed in comparison with data reported from Ethiopia [16] as well as other part of the world [17], even though their isolates were from clinical samples. The present study showed that all the isolates (100%) were multiple resistant to at least three antimicrobials being used. This figure is much higher than earlier reports from different studies in the country [18]. It is also higher than reports of other studies from other parts of the world that showed most of the isolates were found to be sensitive for the antibiotics used [19]. This result revealed that the isolates were highly resistant to Penicillin G, Ampicillin, Streptomycin, Chloramphenicol, Kanamycin and Methicillin. This is in agreement with the work by [20] that they reported high resistance for Penicillin G, Ampicillin and Chloramphenicol. This observation can be attributed in part to earlier exposure of the isolates to these drugs which may have enhanced resistant development [21]. The continuous genetic variation could also have contributed to the increased resistance [22]. Almost all isolates demonstrated resistance to Penicillin G (97.1%) and above 72.5% of the isolates were found to be resistant to Chloramphenicol and Kanamycin. All the isolates of *S.aurues* showed resistant to Streptomycin and Ampicillin and this is an increasing resistance pattern to these antibiotics. This might be due to an important role played by plasmids for the spread of drug resistant organisms [23].This increasing in the Penicillin resistance isolates among Staphylococci strains can be explained in most cases to the production of β-lactamase enzyme that destroyed the β-lactam ring and inactivated the Penicillin antibiotic and this enzyme was encoded by plasmid that easy to transfer among strains [24]. The replacement of the sensitive strains by more virulent or resistant strains and the continuous increment of resistant strains from time to time in kitchen settings might also have contributed to the increased resistance. From the 12 antimicrobial agents used, only Amikacin, Ciprofloxacin and Norfloxacin showed high efficacy against the isolates with the least developed resistance, thus, consistently effective against *S. aureus.*

4. CONCLUSIONS

The high prevalence of *S.aureus* isolated found in this study revealed that kitchen sponges daily used in food establishments and households as well have been recognized as potential agents in the spread of microorganisms, and it has been observed that bacteria persist in these vehicles. The antibiotic sensitivity test on *S.aureus* isolated from kitchen sponges also showed high resistant patterns on some currently prescribed drugs in Ethiopia. There is a need to educate food establishment workers, employers, consumers, and all other individuals who use kitchen sponges frequently about the hygienic practice and appropriate usage of these materials and in practice of indiscriminate use of drugs should be controlled. The carrier state of MRSA in food handlers and food processers as well should be assessed and intervention treatment could be measured.

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COMPETING INTERESTS

The authors declare that there are no competing interests.

AUTHORS' CONTRIBUTIONS

TW carried out the conception of the research concept and design the methodology, carried out the laboratory work, data analysis and preparation of the manuscript for publication. **KB** critically commented and revised the proposal, designed the methodology, carried out the laboratory work and revision of the manuscript. **MA** critically revised the proposal, designed the methodology, and reviewed the manuscript for publication. **HS** carried out data analysis and preparation of the manuscript for publication. All authors read and approved the final manuscript.

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