

EFFECT OF ANTISEPTICS AGAINST STAPHYLOCOCCUS AUREUS ON THE HANDS OF FOOD HANDLERS AND SUSCEPTIBILITY OF PERSISTENT STRAINS TO ANTIBIOTICS

*Uzeh, R. E. and Alayande, T. D.

Department of Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria

*Corresponding author: ruzeh@unilag.edu.ng; roseline.uzeh@gmail.com

ABSTRACT

Effect of antiseptics against Staphylococcus aureus on the hands of food handlers was investigated. The palms of 50 food handlers in restaurants of a tertiary institution in Lagos, Nigeria were swabbed before and after the use of antiseptics and analysed for the presence and population of Staphylococcus aureus. Mannitol Salt Agar (MSA) plates were inoculated with the swab sticks and incubated at 37°C for 24-48h. S. aureus was identified by the production of golden yellow colonies on MSA, Gram positive cocci in bunches that were catalase and coagulase positive, and oxidase negative. A total of 2.2×10^3 cfu/ml/5cm² were obtained before the use of antiseptics and 3.79×10^2 cfu/ml/5cm² after the use of antiseptics. Antiseptics had 83% reduction on S. aureus. Of the two antiseptics used, chloroxylenol (4.8%) had a 69% effect while alcohol gel proved to be more effective with a 90% reduction on S. aureus. A total of 41(82%) out of the 50 food handlers had S. aureus on their palms. The antibiotics susceptibility of S. aureus was investigated. Vancomycin (30µg) was the most resisted antibiotics with 57% followed by 28% resistance against oxacillin (1µ) 18% against cefoxitine (30µ). The food handlers may therefore be considered potential source of multidrug resistant S. aureus in food. We therefore recommend that they use antiseptics on their palms since this helped in the reduction of staphylococcal population.

Keywords: Food handlers, *Staphylococcus aureus*, antiseptics, antibiotics

INTRODUCTION

Members of the Genus *Staphylococcus* are widespread in nature as they can be found in the air, food, dust, water, and on humans and animals. The main human reservoirs are the skin and nasal cavity. About 40 to 44% of healthy humans carry staphylococci in the nose. Food-borne diseases are major global health problem and food handlers play a major role in their transmission. Poor food handling practice give rise to food contamination which subsequently results in numerous food borne diseases (Chekol *et al.*, 2019). It has been established globally that 50% of the total food poisoning cases may be due to improper food handling (Lee *et al.*, 2017). Consumption of food and water contaminated by *Staphylococcus aureus* is of health hazard. The hands of ready-to-eat food service employees have been shown to be implicated in the spread of foodborne disease, mainly because of poor personal

hygiene (Lambrechts *et al.*, 2014). Among the estimated value of about 600 million food borne illnesses that occur each year, 420,000 deaths occur due to poor food handling (WHO, 2015).

Washing of hands with antiseptics is a very vital process of reducing the transfer of bacteria from person to person, person to food or from person to food contact surfaces which may subsequently protect against the spread of diseases (Chinakwe *et al.*, 2012). When food workers visit the toilet, their hands if not properly washed, can transmit pathogens, especially faecal pathogens to food products and as such poor personal hygiene contribute immensely to foodborne illness.

Staphylococcus aureus is the most common species of *Staphylococcus* that can cause infections and is a successful pathogen due to a combination of nasal carriage and bacterial immune-evasive strategies (Cole *et al.*, 2001). *S. aureus* can be transmitted by food handlers who transfer the bacteria from hands to food. The bacteria grow in food and produce enterotoxins which cause illness. The toxins are not heat labile. Protein-containing foods such as meats, poultry, fish, milk, salads made with meat or eggs, puddings, custards, and cream filled pies are foods commonly involved.

Increasing bacterial resistance to antibiotics and antimicrobials is a growing concern facing the medical, food, and sanitation industries (Hola *et al.*, 2002). Methicillin-resistant *S. aureus* (MRSA) is one of a number of greatly feared strains of *S. aureus* which have become resistant to most β -lactam antibiotics. For this reason, vancomycin, a glycopeptide antibiotic, is commonly used to combat MRSA. This pattern of resistance which started in hospitals and later spread to the community, is now well established (Cosgrove *et al.*, 2003). MRSA isolates came into existence soon after the introduction of methicillin. However, in recent years, different strains with unique phenotypes have emerged in the community, and the reservoir of community-associated MRSA is rapidly expanding. In subsequent years, close to 100 cases of *S. aureus* with reduced susceptibility to vancomycin have been reported in the United States (de Lassigne *et al.*, 2006), with some strains responsible for life-threatening systemic infections (Sancak *et al.*, 2005).

As *S. aureus* becomes resistant to more and more antibiotics, many attempts have been made to find other ways to treat infections. In a study performed by Capparelli *et al.* (2007), phage treatment was found successful in treating and saving 97% of mice that were injected with *S. aureus* bacterium and a phage. More methods are

being developed to combat antibiotic resistance because bacteria continue to develop resistance mechanisms (Capparelli *et al.*, 2007).

The aim of this study is to determine the effect of antiseptics on *Staphylococcus aureus* on the hands of food handlers in restaurants of a tertiary institution in Lagos, Nigeria, and susceptibility of the isolates to antibiotics.

MATERIALS AND METHODS

Sample collection: A total of fifty (50) food handlers made up of males and females were randomly selected from ten (10) restaurants located in a tertiary institution in Lagos, Nigeria. They were selected because they are predominantly involved in preparing and serving ready-to-eat foods. After the subjects were thoroughly briefed about the study and given signed consent to participate, a measured portion of each food handler's palm (approximately 5cm²) was swabbed using sterile swab sticks previously moistened with normal saline, after which antiseptics were used to clean their palms. Fifteen seconds after the use of antiseptics, their palms were swabbed again. A total of 100 samples were collected, 25 before/after the use of chloroxylenol (4.8%) and 25 before/after the use of alcohol gel.

Isolation of *Staphylococcus aureus*

The antiseptics (chloroxylenol and alcohol gel) used were first cultured on Nutrient Agar at 37°C for 24-48h to ensure it was not contaminated in any form. Each swab stick used for swabbing palms of food handlers before and after treatment with antiseptics was streaked on Mannitol Salt Agar (MSA) plates and incubated at 37°C for 24-48 h. The plates were observed for growth and colonies counted. The presumptive *Staphylococcus* which grew with golden yellow pigmentation were further sub cultured to obtain pure cultures.

Identification of bacterial isolate

Identification of *S. aureus* was carried out based on the colonial morphology, Gram reaction and biochemical tests (catalase, tube coagulase and oxidase tests).

Antimicrobial susceptibility test

Susceptibility of the bacterial isolates to antibiotics was tested by the disc diffusion method on Mueller Hinton agar. The antibiotics used were ciprofloxacin (CIP) 5µg, cefoxitin (FOX) 30µg, oxacillin (OX) 1µg and vancomycin (VAN) 30µg. Mueller-Hinton agar plates were inoculated with overnight broth culture of the isolates and antibiotic discs were placed on them. The plates were incubated for 24h at 37°C.

The diameter of all zones of inhibition were measured in millimetres (mm) and the values were translated to categories of susceptible, intermediate, or resistant using Clinical and Laboratory Standard Institute (CLSI, 2014).

RESULTS

The isolates appeared as golden yellow colonies as a result of mannitol fermentation. They were catalase positive, coagulase positive and oxidase negative. They were observed to be Gram positive cocci in clusters when viewed under the light microscope.

Staphylococcus aureus strains were isolated from 41 (82%) out of the 50 food handlers in restaurants. The *S. aureus* count before the use of chloroxylenol (4.8%) was 7.53×10^2 cfu/ml/5cm² and 2.31×10^2 cfu/ml/5cm² after usage which resulted in 69% efficacy. *S. aureus* count of 1.447×10^3 cfu/ml/5cm² and 1.48×10^2 cfu/ml/5cm² were obtained before and after the use of alcohol gel respectively, thereby resulting in 90% efficacy (Fig. 1). The total count of *S. aureus* before the use of both antiseptics was 2.2×10^3 cfu/ml/5cm² and 3.79×10^2 cfu/ml/5cm² after use thereby producing 83% effect of the antiseptics against *S. aureus*.

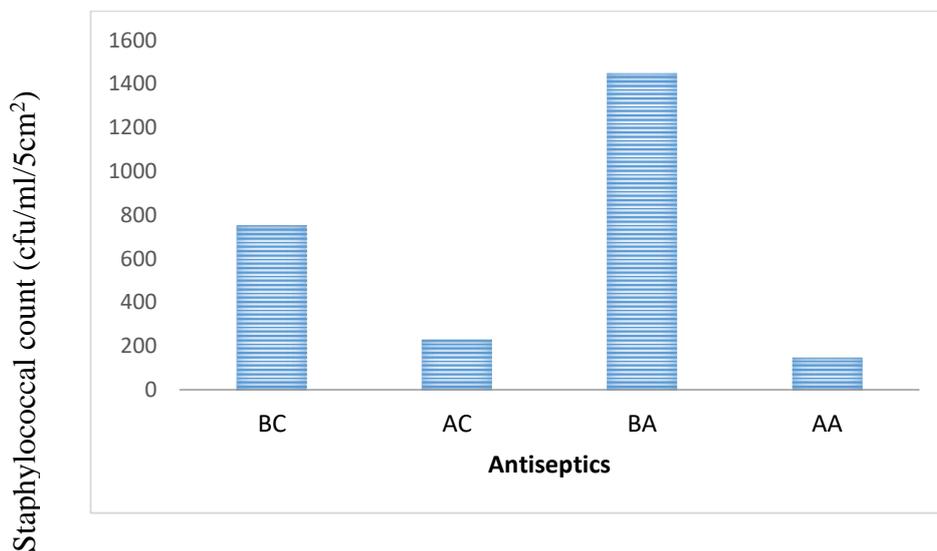


Fig. 1: Staphylococcal count on the hands of food handlers before and after the use of antiseptics

Key:

BC: Before use of chloroxylonol (4.8%)

AC: After the use of chloroxylonol (4.8%)

BA: Before the use of alcohol gel

AA: After the use of alcohol gel

The antimicrobial susceptibility of the *S. aureus* isolates is shown in Table 1. None of the isolates were resistant to ciprofloxacin, 13 isolates (18%) were resistant to ceftiofime, 20 isolates (28%) to oxacillin and 41 isolates (57%) to vancomycin. One isolate (1%) had intermediate resistance towards ciprofloxacin while 15 (21%), 18 (25%) and 15 (21%) isolates had intermediate resistance to ceftiofime, oxacillin and vancomycin respectively. *S. aureus* had the highest resistance (57%) to vancomycin.

Table 1: Antimicrobial susceptibility profile of *Staphylococcus aureus* isolated from the hands of food handlers

Antimicrobial agent	Susceptibility		
	Number of isolates (%)		
	R	I	S
Ciprofloxacin (5µg)	0 (0)	1 (1)	71(99)
Ceftiofime (30µg)	13 (18)	15 (21)	44 (61)
Oxacillin (1µg)	18 (28)	18 (25)	34 (47)
Vancomycin (30µg)	41 (57)	15 (21)	16 (22)

R = Resistance, I = Intermediate, S = Susceptible

DISCUSSION

In this study, 41 out of the 50 food handlers examined were carriers of *Staphylococcus aureus* which makes the percentage of workers carrying *Staphylococcus aureus* on their hands 82%. This is a cause of concern because *S. aureus* can be transferred to food by food handlers and the organism is an important agent of food intoxication. Asymptomatic carriers play an important role in the spread of the organism especially people in professional activities related to public health or food processing (Araujo *et al.*, 2002). In a similar study conducted by Udo *et al.* (1999), they found that 81.61% of the isolates obtained from the hands of food handlers were however coagulase negative *Staphylococcus*. Similar results from isolation of organisms from the hands of food handlers was also obtained by Cepoglu *et al.* (2010) with 72.8% of the organisms obtained being coagulase negative *Staphylococcus*. Castro *et al* (2016) in their study reported 11.1% of *S.*

aureus prevalence on the hands of food handlers. Results may differ due to geographical regions as mentioned by Udo *et al.* (1999).

Foods processed, packaged, transported and stored under poor hygiene stand the risk of being contaminated with infectious or toxigenic microorganisms. Such foods can be a source of illness for humans. One aspect in the investigations of food poisoning outbreaks is to determine how the implicated food becomes contaminated. It is recognized that food handlers are the major source of contamination with *Staphylococcus*. High frequency of carrier status among food handlers has been identified by some investigators. Pereira *et al.* (1994) showed that 30 to 50% of food handlers were carriers of staphylococci. Fifty-five healthy food handlers in a large industrial kitchen in Belo Horizonte (Brazil) were examined and 32 (58.2%) were found to be carriers of *S. aureus* and 17 (30.9%) carried enterotoxigenic strains in their nasal cavity, throat and under fingernails.

In this study, a total of 2.2×10^3 cfu/ml/5cm² *S. aureus* were obtained before the use of both antiseptics while 3.79×10^2 cfu/ml/5cm² were obtained after the use of the antiseptics producing 83% antibacterial effect. Before and after the use of chloroxylenol (4.8%) 7.53×10^2 cfu/ml/5cm² and 2.31×10^2 cfu/ml/5cm² *S. aureus* were obtained respectively leaving the impact of the antiseptics at 69% effect. A total of 1.447×10^3 cfu/ml/5cm² and 1.48×10^2 cfu/ml/5cm² *S. aureus* were obtained before and after the use of alcohol gel respectively, thereby producing a 90% effect. The use of antiseptics helped to reduce the incidence of *S. aureus* on the hands of food handlers. However, the level of antibacterial effect was dependent on the type of antiseptics used.

Among the *S. aureus* 99% of the isolates were susceptible to ciprofloxacin, 61% to ceftiofene, and 47% to oxacillin while 22% susceptibility was observed in the case of vancomycin. However, 57% of the isolates showed resistance to vancomycin, 28% to oxacillin and 18% towards ceftiofene. The multidrug resistance of the organism is of concern because if it gets into food and cause food poisoning when such food is consumed, it will be difficult to treat. Antibiotic resistance by various mechanisms has increased worldwide in bacterial pathogens leading to treatment failures in human and animal infectious diseases (WHO 2007). Castro *et al* (2016) reported that 82% of the *S. aureus* they isolated were resistant to at least one of the antibiotics they tested against the organism. Resistance against antibiotics by pathogenic bacteria is a major concern in the antimicrobial therapy for both humans and animals. Bacteria are able to adapt rapidly to new environmental conditions

such as the presence of antimicrobial molecules, and as a consequence, resistance increases with the antimicrobial use (Jansen *et al.*, 2006; Falagas and Bliziotis 2007). Serious concerns about bacterial drug resistance from nosocomial, community-acquired and food-borne pathogens have been growing for a number of years and have been raised at both national and international levels (Jansen *et al.*, 2006).

CONCLUSION

Food handlers in a tertiary institution in Lagos, Nigeria have been identified in this study to be carriers of *S. aureus* on their hands. Antiseptics however had antibacterial effect on the organism by reducing the count. The persistent isolates had multidrug resistance. This is of public health concern because of the possible hazard the organism can cause in humans through consumption of contaminated foods. We therefore recommend the use of antiseptics by food handlers and maintenance of strict hygiene in order to prevent foodborne disease outbreak.

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