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A Research Note

In vitro Antibiotic Sensitivity of *Staphylococcus aureus* Strains Isolated from a Nigerian Fermented Cereal Drink

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ABSTRACT

The sensitivity of 253 *Staphylococcus aureus* strains isolated from a Nigerian locally-fermented cereal drink (kunun zaki) to 11 antimicrobial agents was determined. Two hundred and forty-seven (97.6%) strains were resistant to one or more of the antimicrobial agents and only 4 (1.6%) isolates were sensitive to all of the agents. Of the isolates from the kunun zaki preparation, 97.8% were resistant while 98.6% from kunun zaki bought at sale outlet and 96.7% of those isolated from utensils were resistant. There was no significant difference in resistance of isolates from the sources ($P>0.05$, X^2). Resistance to penicillin (92.9%), ampicillin (92.9%) and trimethoprim (80.2%) was most frequently encountered while fewer isolates wereresistant to gentamycin (2.0%) and neomycin (3.6%). The predominant antimicrobial resistance pattern was ampicillin-penicillin-trimethoprim detected in 43 (17.0%) strains. The relatively high resistance of *S. aureus* strains isolated from kunun zaki to the tested antimicrobial agents is suggestive of misuse of those drugs in Nigeria.

Staphylococcus aureus is distributed widely in nature having been isolated from human beings, a variety of animal species and food items, raw and ready-to-eat *(1,10,16)*. Staphylococci have also been demonstrated to cause many human and animal diseases *(8,17)*. In an effort to control some of these diseases through use of antimicrobial agents in prophylaxis and therapy, misuse has resulted in gradual increase in the prevalence of resistance to some of these agents *(1,10)*.

In Nigeria, like in other developing countries, little or no control exists in the dispensation and use of antimicrobial agents, particularly antibiotics. These are readily available in stores from medically unqualified attendants. It was therefore hardly a surprise to find that a high percentage of staphylococci isolated from humans and animals in Nigeria were resistant to commonly used antimicrobial agents in human and veterinary practice *(1,16)*. Similarly, ready-to-eat products of animal origin, but exposed to human handling, were found to contain a high number of staphylococci *(3)*. A high prevalence of resistance to antibiotics was also detected amongst staphylococcal isolates from these products *(10)*.

Kunun zaki is a popular locally-fermented cereal product consumed in northern Nigeria, particularly during the hot months of the year. In the preparatory stage of the products, materials are exposed to a lot of human handling under insanitary conditions *(12)*. The prevalence of staphylococci in kunun zaki and their enterotoxigenicity were recently reported *(12,13)*.

In an on-going effort to monitor the antibiotic resistance patterns of staphylococci from various sources in Nigeria, this study determined the antibiograms of isolates from a cereal drink based on plant products (cereals) and exposed to human handling.

MATERIALS AND METHODS

Kunun zaki is prepared from guinea-corn, millet or maize which is soaked in water for 1-2 d and then ground into flour at public mills. A paste of the flour is made in cold water. Boiled water is added to one half of the paste, resulting in a homogenous or semi-solid form. Cold water is added to the other half and this is subsequently mixed with the homogenous half. Various spices in powder form (ginger, black pepper, cloves and red pepper) already mixed in water are passed through either a nylon piece of cloth or a metallic sieve and added to the homogenous mixture. Ground sugar in water is also passed through the nylon sieve and added to taste. Finally, the product is bottled as kunun zaki and ready for consumption. These are hawked around or sold at some kiosks.

Six points of preparation and six sale outlets (local kiosks and hawkers) for kunun zaki were randomly selected in Samaru to serve as sources for the product. Approximately 100 ml of kunun zaki was obtained from each source weekly over 3 months.

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Serial dilutions of kunun zaki in saline solution used for quantitation of staphylococci *(12)* were plated on Baird-Parker agar (BPA). The plates were incubated at 37°C for 48 h.

Representative black colonies on BPA were picked at the various dilutions and those that were catalase-positive and gram-positive cocci were streaked on nutrient agar slants until needed.

The ability of isolates to utilize mannitol and glucose aerobically and anaerobically was detected as earlier described *(19)*. Production of acid in 5 d at 37°C was considered as a positive reaction.

The tube coagulase test was performed using freshly prepared rabbit plasma on an overnight culture in brain heart infusion broth at 37°C. The procedure described by Baer et al. *(6)* and test interpretation protocol of Sperber and Tatini *(18)* were used. The test procedure of Lachica et al. *(11)* was used to detect thermonuclease production.

For the study, staphylococcal isolates that were gram-positive cocci, catalase-positive, utilized glucose and mannitol anaerobically, coagulated rabbit plasma or produced thermonuclease were considered to be *S. aureus*.

To determine the susceptibility of *S. aureus* strains to antimicrobial agents, the following antibiotic discs (Difco) and concentrations were used: penicillin (10 units), erythromycin (15 μg), streptomycin (10 μg), tetracyclinc (30 μg), methicillin (5 μg), neomycin (30 μg), trimethoprim (5 μg), chloramphenicol (30 μg), gentamycin (10 μg), ampicillin (10 μg) and bacitracin (10 units).

The disc diffusion method described by Bauer et al. *(7)* was used. Approximately 5-6 antimicrobial agent discs were placed on each inoculated Mueller-Hinton agar plate. Inoculated plates were incubated at 37°C overnight in the inverted position. Zones of inhibition measured to the nearest millimeter were interpreted as susceptible and resistant based on the table recommended by the disc manufacturer.

RESULTS AND DISCUSSION

Of the 253 staphylococcal isolates tested, 247 (97.6%) were resistant to one or more of the 11 antimicrobial agents used (Table 1). At the point of preparation, the prevalence of resistance of *S. aureus* strains isolated from kunun zaki was 97.8% while those obtained at the sale outlets it was 98.6%.

TABLE 1. *Resistance of* S. aureus *strains from kunan zaki and utensils to one or more antimicrobial agents tested.*

Source of sample	Type of sample	No. of isolates tested	No. (Percent) resistant
Point of preparation	Kunun zaki	90	88 (97.8)
	Utensils	91	88 (96.7)
Point of sale	Kunun zaki	72	71 (98.6)
Total		253	247 (97.6)

The distribution of antimicrobial agent resistance of *S. aureus* isolates is shown in Table 2. The prevalence to ampicillin (92.9%) and penicillin (92.9%) were highest while gentamycin and neomycin appeared to be most effective on isolates with values of 2.0% and 3.6%, respectively. Generally, the prevalence of resistance by isolates to drugs used was similar for those from kunun zaki and utensils at points of preparation. For staphylococcal isolates from kunun zaki alone, for those from points of preparation, fewer were resistant to erythromycin (17.8%), methicillin (38.9%) and streptomycin (16.7%) compared to those from the sale outlets, 52.8%, 98.6% and 86.1%, respectively.

The predominant antimicrobial resistance patterns were ampicillin-penicillin-trimethoprim (17.0%), ampicillin-bacitracin-erythromycin-methicillin-penicillin-streptomycin-trimethoprim (5.9%) regardless of source. Overall, 78 antimicrobial resistance patterns were observed. Four (1.6%) isolates were sensitive to all the antimicrobial agents tested.

This is considered the first report on the resistance of staphylococci from a Nigerian cereal (plant) product exposed to human handling during preparation. Previous studies have reported on staphylococci from clinical cases in animals and man *(1,16)* and meat products *(10)*. The prevalence of resistance (97.6%) detected in the present study is rather alarming when compared to previous re-

TABLE 2. *Distribution of antimicrobial resistance by* S. aureus *isolates from various sources.*

		No. (%) of *S. aureus* from sources resistant to antimicrobial agents			
		Point of preparation		Point of sale	
Antimicrobial agent	Concentration	Kunun zaki	Utensils	Kunun zaki	All source
Ampicillin	10 μg	87 (96.7)	78 (85.7)	70 (97.2)	235 (92.9)
Bacitracin	10 units	25 (27.8)	43 (47.3)	32 (44.4)	100 (39.5)
Chloramphenicol	30 μg	9 (10.0)	22 (24.2)	29 (40.3)	60 (23.7)
Erythromycin	15 μg	16 (17.8)	33 (36.3)	38 (52.8)	87 (34.4)
Gentamycin	10 units	1 (1.1)	2 (2.2)	3 (4.2)	5 (2.0)
Methicillin	5 μg	35 (38.9)	39 (42.9)	71 (98.6)	145 (57.3)
Neomycin	30 μg	2 (2.2)	2 (2.2)	5 (6.9)	9 (3.6)
Penicillin	10 units	88 (97.8)	79 (86.8)	68 (94.4)	235 (92.9)
Streptomycin	10 μg	15 (16.7)	34 (37.4)	62 (86.1)	111 (43.9)
Tetracycline	5 μg	34 (37.8)	36 (39.6)	25 (34.7)	95 (37.5)
Trimethoprim	5 μg	73 (81.1)	68 (74.7)	62 (86.1)	203 (80.2)
Total number of Isolates		90	91	72	253

ports in Nigeria *(1,10,16)*. This is of health concern as it appears that development of resistance amongst staphylococci is on an increase when compared with a value of 60.3% recorded for staphylococci from ready-to-eat products only a few years ago *(10)*. The type of products and degree of exposure to human handling cannot, however, be ignored. It is pertinent to observe that similar types of antimicrobial agents and concentrations were used in both studies. The wide-spread development of resistance amongst staphylococci in Nigeria may be explained in part by the common interchange of staphylococci between animals and humans *(2,5)* in Nigeria, coupled with poor hygienic practices during preparation and sale of some locally available foods *(4)*.

The relatively high prevalence of resistance to ampicillin and penicillin agrees with previous reports in Nigeria *(1,10,16)*. The ready, uncontrolled availability of these two antibiotics at relatively affordable prices in the population is a major factor for the occurrence of the resistance. Trimethoprim, over the years in Nigeria, has also gained acceptance amongst people in a mixture with sulfamethozazole as Septrin^{R}, and its increased misuse may account for the high prevalence of resistance detected.

It was no surprise to observe that gentamycin was most effective against all isolates tested. This can be explained, in part, by the practice of prescribing the antibiotic to only serious cases in Nigerian hospitals. In addition, it is not readily available in local drug stores and even when obtainable, is at exhorbitant cost beyond the reach of most people.

Of interest is the finding that 57.3% of tested staphylococci from all sources were resistant to methicillin. This is because methicillin is not commonly used in medical and veterinary practice in Nigeria. Ojo *(14)*, in a study at western Nigeria, first reported the resistance of two strains of *S. aureus* to methicillin and felt that the finding may not be unconnected with the use of penicillin in the country. The high prevalence of resistance (92.9%) to penicillin found in the present study supports this thinking. Parker and Hewitt *(15)* found that strains resistant to methicillin were also resistant to at least one antibiotic other than penicillin and methicillin. This was confirmed in the present study where no staphylococcal isolates were resistant to methicillin alone and in the 21 resistance patterns involving methicillin, 3 to 8 other antibiotics were involved.

The high occurrence of multiresistance to antibiotics by isolates should be of concern as it indicates that little or no effort has been made to control the use of these antimicrobial agents in Nigeria, and may constitute a health hazard. The high prevalence of multiple resistance also suggests that resistance plasmids carrying several genes for antibiotic resistance are widespread among the strains. With penicillin and ampicillin most commonly encountered in the patterns, use of penicillinase-resistant penicillin may reduce emergence of resistant variants in Nigeria, as suggested by Bulger and Sherris *(9)*.

In conclusion, the high prevalence of resistance to various antimicrobial agents should be of health concern to necessitate enactment of laws to regulate use of antibiotics in Nigeria. This is because consumers of kunun zaki are exposed to *S. aureus* strains which are resistant to some antibiotics used in the treatment of various diseases in Nigeria.

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non-inhibitory activity exhibited by the cold water extracts from fresh olives could be due to low solubility of the inhibitor(s) in cold water, or the amount of the inhibitor may be much less than in frozen olives. Alternatively, the inhibitor may either be generated, activated or released as a result of freezing.

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